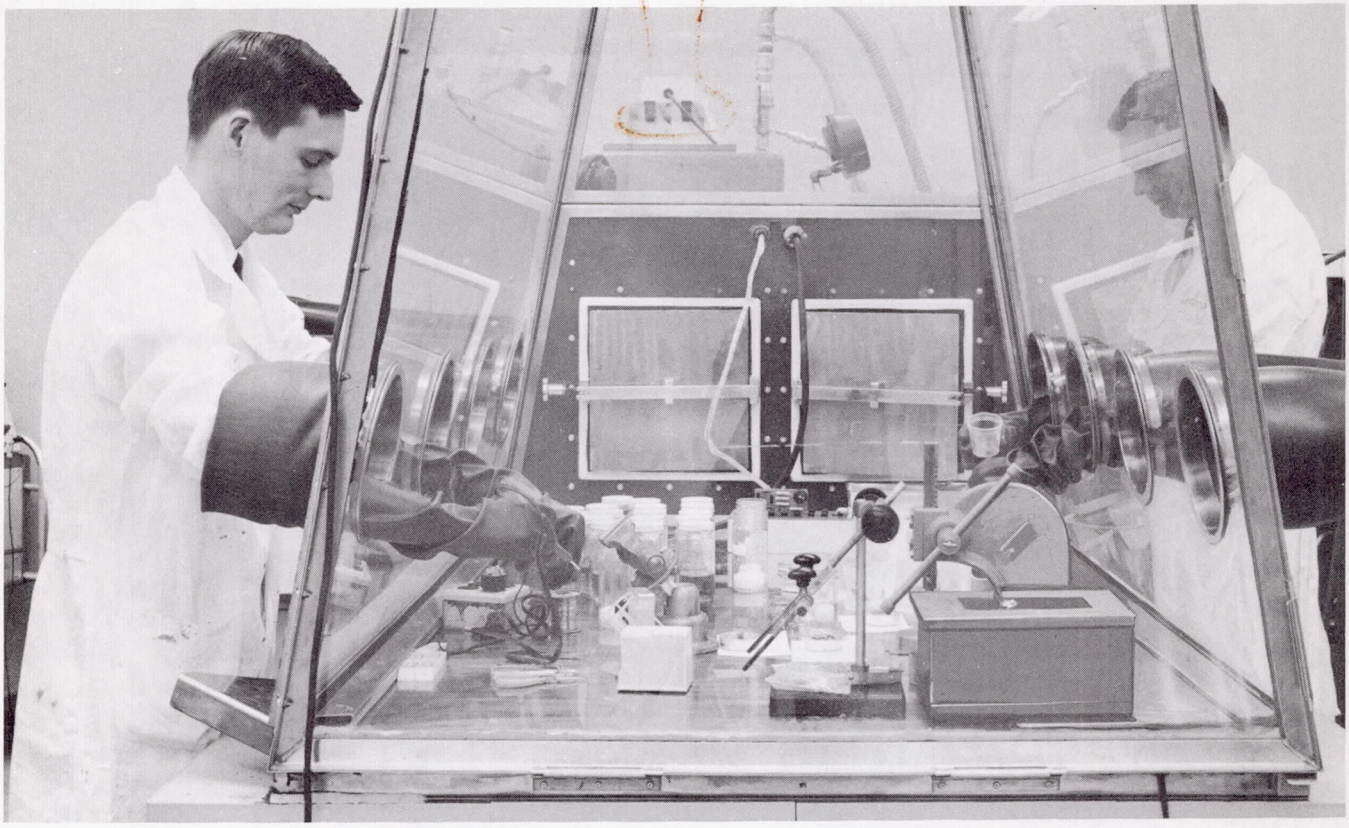
1727

M-56-65-1

21 MARCH 1965



EXPERIMENTAL STUDY OF STERILE ASSEMBLY TECHNIQUES

VOLUME I - FINAL REPORT

CONTRACT NO. JPL 950993
PREPARED FOR CALIFORNIA INSTITUTE OF TECHNOLOGY,
JET PROPULSION LABORATORIES • PASADENA, CALIFORNIA

LOCKHEED MISSILES & SPACE COMPANY
A GROUP DIVISION OF LOCKHEED AIRCRAFT CORPORATION
SUNNYVALE, CALIFORNIA

Page intentionally left blank

FOREWORD

This report covers work accomplished by Lockheed Missiles & Space Company on the Experimental Study of Sterile Assembly Techniques (Contract JPL 950993) for the Jet Propulsion Laboratory, Pasadena, California, under the technical direction of Dr. J. J. McDade of JPL.

The study final report is divided into 15 sections as follows:

- The first section introduces the study and presents background information.
- Section 2 summarizes the study results and presents conclusions and recommendations.
- The next two sections cover the initial phases of the contract, modifications of equipment, and biological materials and methods.
- The next 10 sections, 5 through 14, cover the planned phases of the study, including the various process tests and the assembly of the test circuit under sterilizing conditions.
- The final section, 15, covers the special tests conducted to determine the causes of contamination which occurred during the program.

Three appendixes are provided. Appendix A contains the bacteriology reports compiled during the program. Appendix B is the rough narrative log which covers, on a day-to-day basis, the work conducted in the Sterilization Laboratory. Appendix C contains the rough log sheets maintained for the operations of the sterilization equipment.

Sections 1 through 15 and Appendix A are grouped in this volume, Volume I. Appendixes B and C are included under separate cover as Volume II.

Principal contributers to the study and to this report were:

W. Hansen	Space Programs Office, Project Leader
S. R. Hawes	Space Programs Office
Dr. V. Lynch	Bioastronautics
R. Zeits	Bioastronautics
A. K. Miller	Bioastronautics

CONTENTS

*Appendixes B and C are presented under separate cover as Volume II of this report.

ILLUSTRATIONS

TABLES

Section 1
INTRODUCTION

1.1 BACKGROUND

Voyager spacecraft will initiate exploration of Mars, in accordance with present plans, within the next 10 years. One of the principal goals of these exploration missions will be the search for life. The possibility that such life exists imposes upon any space vehicle which may impact the Martian surface a requirement for absolute sterility. This requirement is necessary (1) to insure that experiments detect only indigenous life rather than life transported from earth by the spacecraft and (2) to prevent biological contamination of the planet by earth organisms which could wreak havoc on Martian life forms if they exist.

The sterility requirement is significantly more severe than those employed for medical or industrial operations on earth. The National Aeronautics and Space Administration goal for Mars landings is that the probability that a single viable earth organism may land on the planet must be less than 1 in 10,000. Dry-heat sterilization of the assembled, shrouded spacecraft is the most attractive method of sterilization. It is possible, however, that all components and systems to be incorporated in a sophisticated Mars landing vehicle will not be capable of withstanding the heat-sterilization cycle.

The Experimental Study of Sterile Assembly Techniques was initiated to explore one practical alternative to total heat sterilization – aseptic assembly of the spacecraft and its heat-sensitive subsystems. This approach would permit each subassembly which could not be sterilized by heat to be sterilized by the most effective method which would not cause operational degradation. The final assembly operation would then be accomplished aseptically in a sterile or sterilizing atmosphere by operators isolated from the sterile environment – through the use of gloves, gas-tight suits, remote

manipulators, or a combination of techniques. The spacecraft would be sealed in a shroud within the sterile atmosphere and thus would remain in a sterile environment until the shroud is separated from the spacecraft after earth escape.

The study was designed around the assembly of an electronic circuit whose manufacture utilizes several of the operations and processes likely to be encountered in the assembly of a Mars landing spacecraft. The use of an oscillator circuit as the major test item also permitted the incorporation of electronic automatic checkout equipment to measure and monitor the effects of the sterilant gas and assembly techniques on the circuit components.

Specific objectives of the study were to determine:

(1) The feasibility of carrying out the assembly of a small electronics unit using glove box techniques
(2) The efficacy of the sterilant gas conditions (a mixture of ethylene oxide, 12 percent by weight and Freon 12, 88 percent by weight*) and time cycle for absolute sterility – using typical system components as controls and a known bacterial spore preparation
(3) The effect of assembly in pure ethylene oxide, sterile nitrogen, and sterile air on sample soldering, bonding, staking, and nut-bolt assembly procedures
(4) The effect of assembly in ethylene oxide, sterile nitrogen, and sterile air on a complete unit
(5) The effect of assembly in ethylene oxide, sterile nitrogen, and sterile air on the performance and/or reliability of the test unit
(6) The effect of operation in ethylene oxide, sterile nitrogen, and sterile air on the performance and/or reliability of the test unit
(7) The efficacy of a sterile sealing system used for the unit

The methods used to achieve these objectives are presented in the sections to follow.

---

*Throughout this report, ETO is used as an abbreviation for the ethylene oxide/Freon 12 mixture specified. Pure ethylene oxide is designated simply as "ethylene oxide."

1.2 GENERAL APPROACH

The study program plan approached the achievement of these objectives in a systematic manner. The individual processes contemplated for use in the assembly of the complete circuit, as well as additional typical electronic assembly operations, were first tested individually for the effects of the gas atmosphere and assembly techniques, and from the standpoint of the achievement of sterility. These processes were hand soldering, dip soldering, nut and bolt connecting, staking (terminal attachment), epoxy bonding, and potting. Control processes on the bench duplicated each operation conducted in the gas atmosphere. A glove box system was used to contain, and permit work in, the various atmospheres.

Next, a breadboard of the printed circuit card – consisting of all circuit components hand soldered to terminals – was assembled on the bench and checked out with the automatic checkout equipment. The breadboard circuit was then disassembled by unsoldering the leads, allowed to soak in the gas atmsophere, reassembled in the gas, and again checked out.

The next phase of the program was the assembly of the circuit on a printed circuit card – first in ETO, then in sterile air, and finally in sterile nitrogen. As in the case of the breadboard circuit, automatic checkout equipment was used to monitor circuit performance through all stages of manufacture. Completed, checked-out circuits were then cut up and placed in jars of nutrient to check for viable organisms.

In the program's final stage, completed printed circuit cards were sealed in gasketed aluminum cans in the environment in which they were assembled – i.e., laboratory air, ETO, sterile air, or sterile nitrogen. A gas-tight connector in the can lids permitted the automatic measurement of circuit performance at any time. After sealing, the cans were removed from the glove box system and tested on the bench. The cans were then returned to the glove box system and opened, and the cards were cut up for biological assay.

In all of these stages, the biological portion of the testing was accomplished in the following manner. The test specimen was first heat-sterilized and then inoculated with a large number of B. globigii, an organism highly resistant to ETO. After ethylene oxide sterilization and assembly operations, each test sample was examined to determine whether any of the test organisms remained in a viable form. The bacteriological contamination and testing phase was performed with complete independence from the assembly and electrical testing phase. Rigorous inoculation and assay procedures were employed to provide a maximum challenge to the ETO sterile assembly techniques.

Section 2

SUMMARY OF RESULTS AND CONCLUSIONS

2.1 GENERAL RESULTS AND CONCLUSIONS

The assembly operations, tests, and experiments conducted during the Experimental Study of Sterile Assembly Techniques produced results of significant interest to both the engineering and biological communities; to accomplish the study, it was necessary to interlace the techniques and procedures of both to their mutual benefit. Much definitive information concerning the feasibility of sterile assembly techniques was developed and areas for further investigation identified.

The major goals of the program were achieved. General results and conclusions are as follows:

- Assembly of electronic circuits in atmospheres of ethylene oxide/ Freon 12 (ETO), sterile air, and sterile nitrogen using glove box techniques is feasible. Glove box work increases the time required to achieve the same product quality by an approximate factor of 3.
- The individual processes of hand soldering, dip soldering, staking, nut and bolt connecting, epoxy bonding, and potting can be accomplished in an ETO atmosphere without harmful effect on the component.
- The performance of a complete unit assembled in ETO, sterile nitrogen, or sterile air is not significantly different from the performance of a unit assembled in a normal laboratory environment.
- The performance of a unit sealed in a can and operated in atmospheres of ETO, sterile nitrogen, or sterile air is not significantly different from the performance of units assembled and packaged in the laboratory atmosphere.
- The maintenance of a component or unit's sterility through aseptic assembly in ETO is feasible. However, the effectiveness of ETO in sterilizing some

of the typical spacecraft components and materials used in the program was not demonstrated.

Major areas in which the results of the study indicate that additional investigations should be accomplished are:

(1) The relationship between material surfaces and the ability of ETO to sterilize
(2) The further determination of the effects of ETO, relative humidity, spore hydration, and spore rehydration on ETO sterilization
(3) The effects of high temperature on the ethylene oxide/ Freon mixture and the effects of resulting chemical reactions on tool and component materials
(4) The feasibility of combining various sterilizing techniques followed by aseptic assembly in ETO to achieve and maintain sterility
(5) The development of sterilization assay procedures for components which have toxic reactions with test nutrients

2.2 EQUIPMENT DESIGN RECOMMENDATIONS

Recommendations relative to the design of glove box systems and equipment for sterile assembly operations are as follows:

- Incorporation of a flexible top or other design to absorb pressure fluctuations caused by entry into, motions while working within, and exit from the glove box. A large working volume damps these fluctuations.
- Use of butyl rubber gloves of at least 0.03-in. thickness for working in ETO atmospheres. This permits operating without time limitations.
- Incorporation of an electronic humidity-indicating system rather than a measuring device which introduces water vapor into the gas atmosphere
- Use of a simple system for the introduction of ETO into the assembly chamber. If a pressure regulator is incorporated, it should be installed at a point where no liquid ETO can reach the inlet.
- Use of a system which introduces water vapor into the assembly area volume rather than into the incoming gas stream

- Incorporation of a gas circulation system within the assembly chamber
- Use of a suspension system for components awaiting assembly in the chamber, to assure moving gas contact with all surfaces
- Incorporation of a positive gas-relief safety system which will prevent inadvertant over-pressurization
- Incorporation of a positive ethylene oxide concentration detection system which measures concentration within the chamber, rather than the concentration of the effluent
- Design for a maximum working pressure of 2-in. H_2O or less for comfortable working conditions. The chamber design should also permit an operator to work comfortably in a sitting position.
- Provision of special fixtures and tools to reach, transfer, and hold assembly items within the box. They should be simple devices requiring simple operational motions, and should occupy little space.

2.3 OPERATIONAL RECOMMENDATIONS

Recommendations relative to operations required during sterile assembly are:

- All steps to be conducted within the assembly chamber should be planned thoroughly so that all necessary tools and equipment are present in the chamber when operations start. A dry run outside the assembly chamber is strongly recommended.
- When conducting assembly operations which involve pressure increases—such as system charging and dip soldering—two persons should be present, one to perform the operation and one as a safety observer.
- The assembly chamber should be purged with ETO after operations involving high temperatures, such as dip soldering. A daily purge is also desirable.

2.4 CIRCUIT ASSEMBLY RECOMMENDATIONS

The following recommendations apply to assembling printed-circuit cards in a glove box in ETO:

- The bottom of each heavy fixture (vises, swaging machines, etc.) should be weighted, as permanent attachment to the floor of the glove box is impractical.
- All hand tools should have non-slip grips for glove work.
- A small brush and dust pan should be included in the inventory of tools to be placed in the sterilizing chamber; they should be used frequently to maintain the cleanliness of the chamber.
- Soldering irons and other heated units should use sealed elements. The ETO attacks bare wire at high temperatures.
- Soldering iron tip cleanliness is a problem if the tip does not rest in a holder with a small heated pot. The pot also insures good tinning of the tip at all times.
- Flux used in the soldering operation should be a non-conducting type, preferably having insulating properties. Its characteristics should make it possible to remove the flux, using alcohol or similar solvent, from the item being hand- or dip-soldered. If water is used to remove the flux, the humidity in the chamber will quickly rise above sterilizing specifications.

2.5 BIOLOGICAL RECOMMENDATIONS

Specific recommendations which resulted from the study and are relative to the biological techniques and procedures are for use of:

- B. globigii as a test organism in ETO sterilization experiments. Use of this organism is strongly endorsed. Standard laboratory techniques maintained a stable concentration of spores throughout the program.
- The water-dip technique of component inoculation as a more rigorous method than the application of an acetone spore suspension to the item being inoculated
- Tripticase soy agar plating as the technique for determining sample contamination. This was the ultimate and most reliable of the three techniques used

during the study. Identification of the organism is facilitated by its vivid orange pigmentation and the characteristic shape of its colonies.

- Agitation during incubation to assure growth in a minimum length of time
- Seventy-two hours as the incubation and agitation time when testing for growth. No growth was detected in 7 days which had not already been detected after 72 hours.
- As many growth test containers as possible for the sterile assay testing. If possible, each piece part of an assembly should be bottled in an individual container so that contamination can be localized to an individual component or procedure.
- A short agitation and incubation period. Use of a short period is especially important when the component being assayed is toxic, as evidenced by control specimens. If the component is highly toxic, consideration should be given to washing the component in the media, followed by its immediate removal.

Section 3
EQUIPMENT

3.1 STERILIZATION EQUIPMENT

The sterilization and assembly glove-box system used in the study consisted of a main glove box (MGB), a small glove box (SGB) connected to the main box via an interchange lock, and an entry lock connected to the main glove box. A sterilization cart, originally designed to supply ETO and sterile nitrogen to the early Ranger vehicles' shrouds for surface sterilization, provided sterile nitrogen, sterile air, and, at the commencement of the program, ETO to the glove system. Descriptions, modifications performed, and use of these units are discussed in following paragraphs.

3.1.1 Glove Box Systems

The main glove box (MGB) shown in Fig. 3-1 was built by LMSC specifically for sterile assembly operations using Ethylene Oxide/Freon 12 sterilant (ETO). The specifications of the MGB are:

- Dimensions: 6 ft × 4 ft × 4 ft
- Capability: 4 work stations (8 glove ports)
- Maximum pressure: 5 in. H_2O
- Inlet and exhaust ports: 2 provided
  - 1 port at bottom of box
  - 1 port at top of box

Additional features are:

- A baffled gas inlet plenum to allow even filling of box with minimum turbulence
- A large hinged door for entry of large equipment; an inflatable rubber seal around the door minimizes leakage

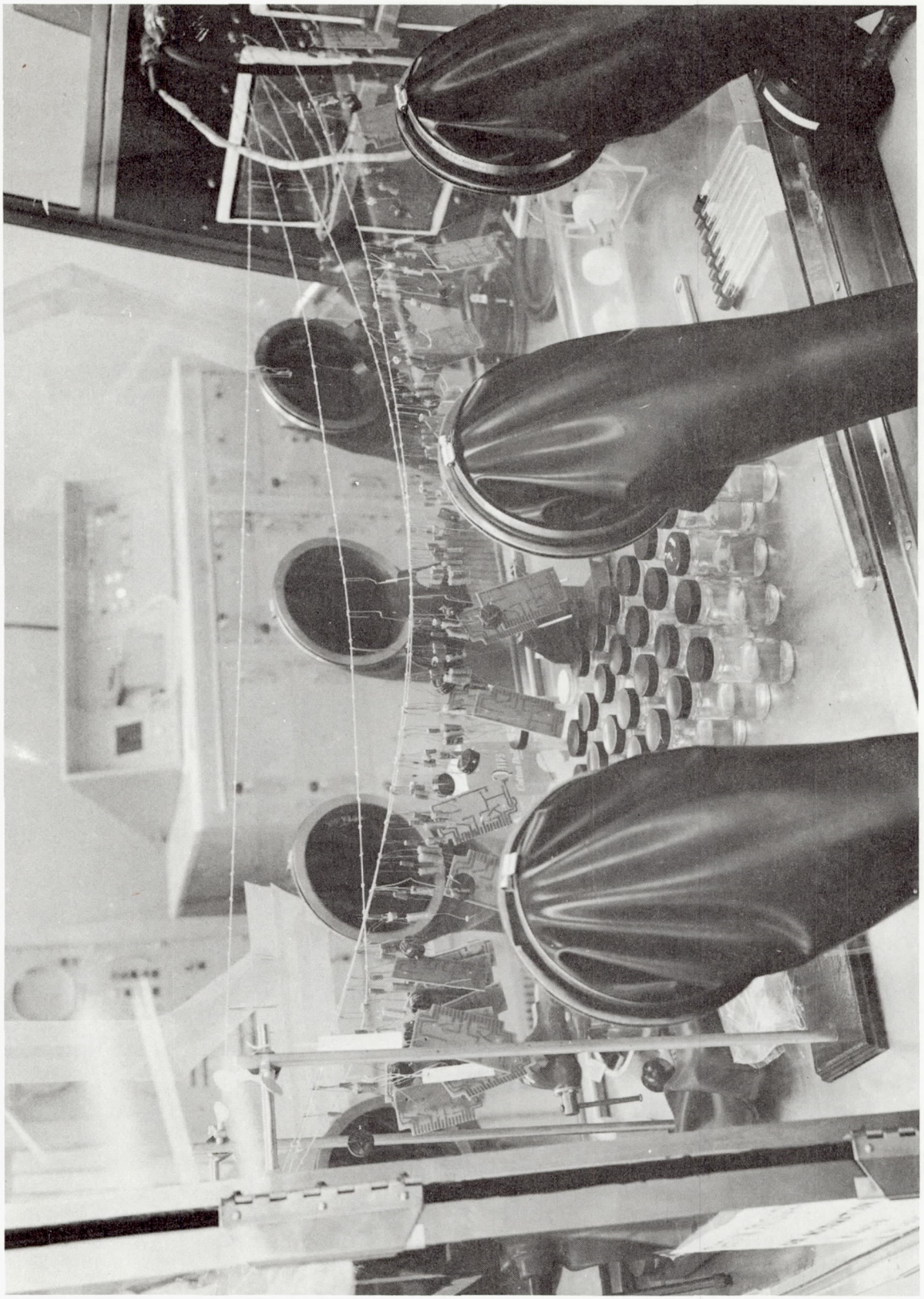


Fig. 3-1 Main Glove Box (MGB)

- A flexible plastic top to accommodate pressure fluctuations as arms are inserted in gloves; design also provides emergency blowout in event of extreme overpressure

The small glove box (SGB) and locks were purchased from General Technology Corporation. The SGB shown in Fig. 3-2 was used for bottling sterile assay samples, and for sterile air and sterile nitrogen assembly. The locks were used to transfer materials from location to location while maintaining atmosphere integrity. The specifications of these items are:

SGB dimensions: 35 in. × 26 in. × 30 in.
Lock dimensions: 14 in. × 14 in. × 20 in.
SGB capability: 1 work station (2 glove ports)
Maximum design pressure: 6 in. H_2O
SGB and lock gas inlet ports: 2 provided at top of each
SGB penetration: multi-contact instrumentation connector and two ac, 115-v power plugs

The gloves installed in the MGB and SGB were the Charleston Rubber Company's "Butasol" brand. Glove thickness was 0.030 inches; no problems were experienced in working with this gauge thickness. This glove proved to be an excellent barrier to ethylene oxide gas.

3.1.2 Sterilization Cart

The sterilization cart is shown in Fig. 3-3. The cart is a mobile unit of ground-support equipment originally designed for use at Cape Kennedy to surface-sterilize the Ranger spacecraft.

Prior to study-contract award, the cart was replumbed by LMSC to permit its use for supply of ETO, sterile nitrogen, and sterile air to the glove box system. A piping diagram of the unit as replumbed at the time of contract award is shown in Fig. 3-4. The functions of the principal cart components are outlined in the following paragraphs.

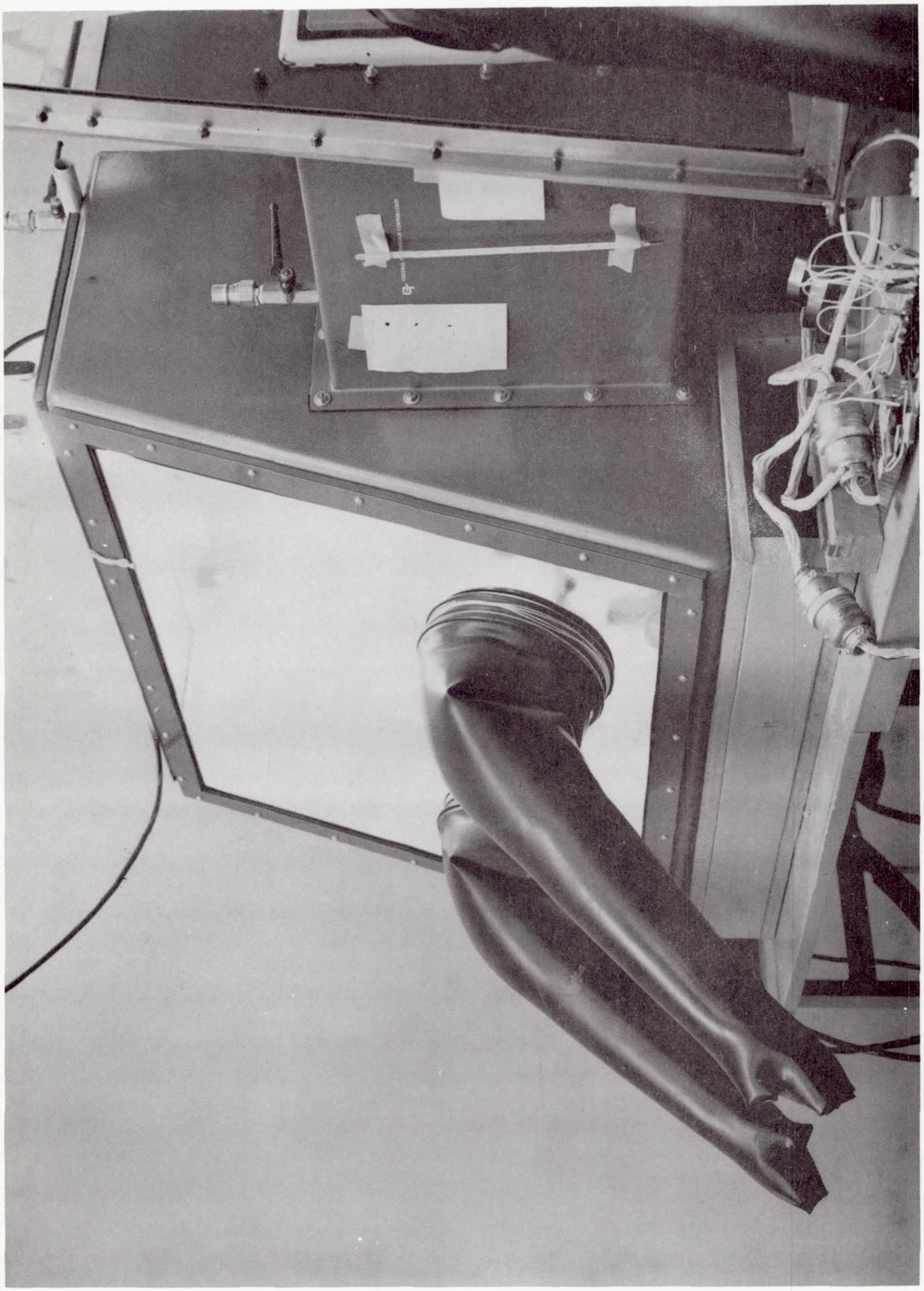


Fig. 3-2 Small Glove Box (SGB)

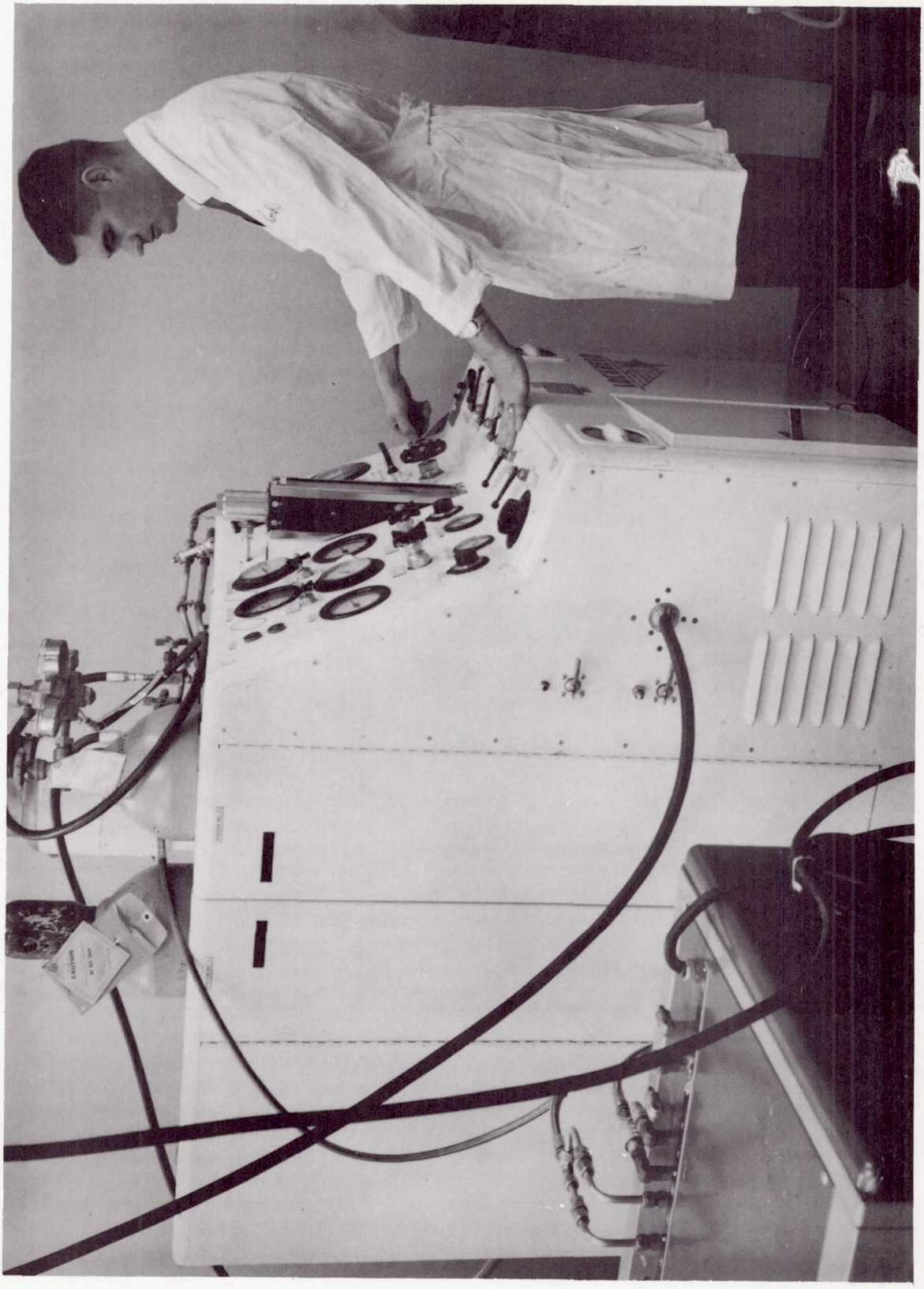


Fig. 3-3 Sterilization Cart

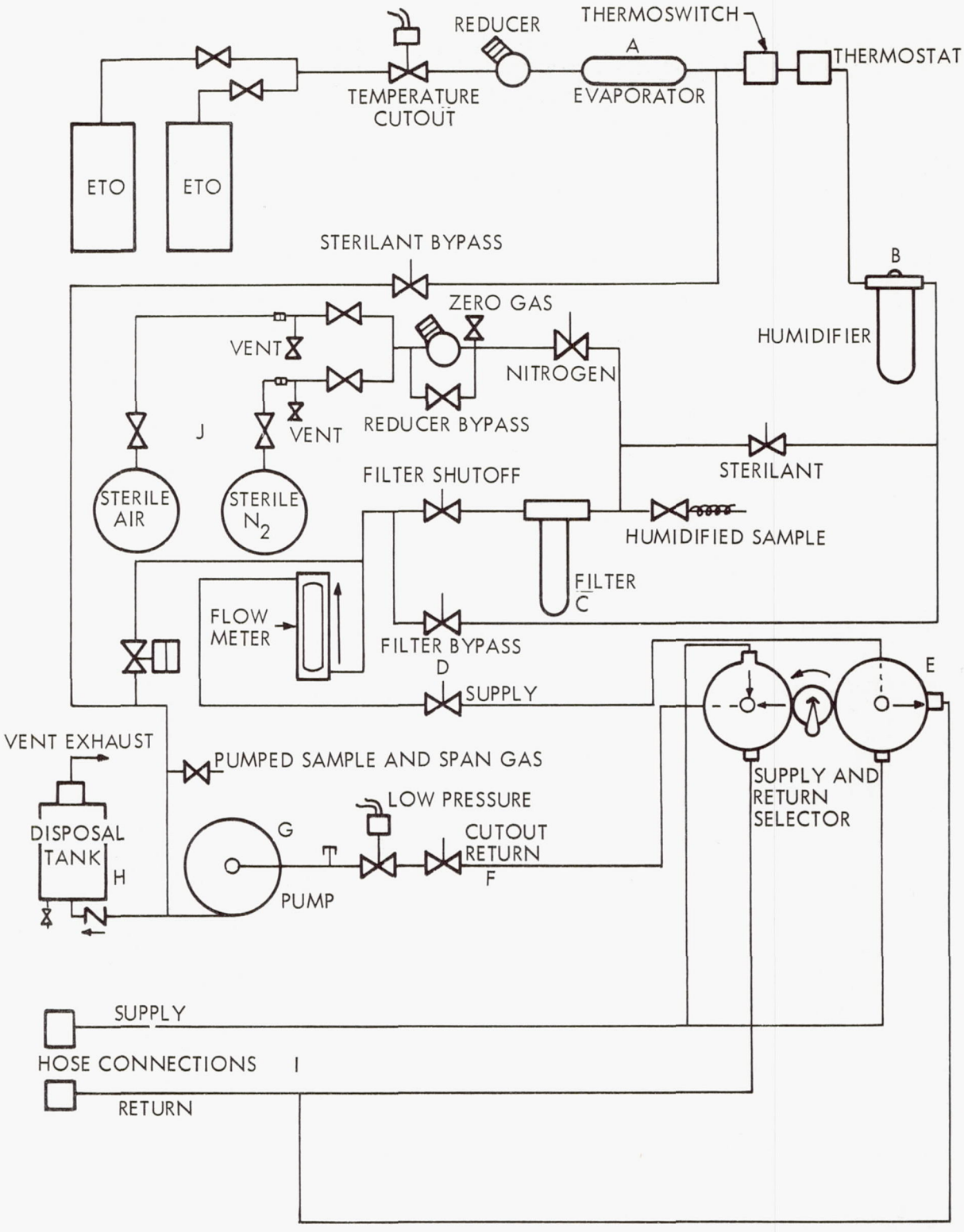


Fig. 3-4 Sterilization Cart Piping Diagram

Evaporator (A). The evaporator vaporizes the liquid ethylene oxide and Freon 12 supplied from either one of two flasks.

Humidifier (B). The humidifier inserts a controlled quantity of water vapor into the ETO gas supply for the glove box system. The unit is actually a modified air-line oiler model 23408-4LC, manufactured by C. A. Norgren Co. Two controls are provided: (1) temperature control of the water, and (2) an orifice control to determine the quantity of water delivered to the gas-supply stream.

Filter (C). The filter is a Pall Corporation microfilter model MCS1001UVH (utilizing UV16H filters with a rating of 0.08 microns) which filters, at the biological level, both the ETO and sterile gases supplied to the glove box system.

Supply valve (D). This is the main valve that controls gas supply to the glove box system. It is mounted on the control panel of the sterilization cart.

Supply and return selector valves (E). These quick throw plug valves permit the ETO supply and return line, and the sterile gas supply and return lines, to be quickly interchanged by moving one selector valve.

Return valve (F). This valve controls suction from the glove box system, and is mounted on the control panel of the sterilization cart.

Vacuum pump (G). This pump takes a suction from the glove box system and discharges to the gas disposal tank.

Disposal tank (H). The disposal tank is filled with water and a small amount of hydrochloric acid. The gas discharge from the vacuum pump bubbles up through the tank. A discharge unit at the top of the tank is connected to atmosphere (lab roof) via a flexible hose. The water in the disposal tank absorbs all ethylene oxide gas in the effluent, changing it to ethylene glycol.

Hose connections (I). The hose connections have female quick disconnect fittings which attach to male fittings on the compartment in the glove box system. When gas is being supplied through one hose the other is lined up to discharge through the return valve, vacuum pump, and disposal tank.

Sterile air and sterile nitrogen flasks (J). These flasks were purchased from Cole Engineering Company of Hayward, California (see Fig. 3-5). They are designed to produce and supply sterile nitrogen and sterile air to the glove box system via the sterilization cart. The tanks are charged to 100 psig with nitrogen or oil-free air, and then heated to 150°C in an oven for at least 24 hours. They are then connected to the sterilization cart. Sterilization of the point where the tank connects to the cart is accomplished by backflow of ETO. The tanks are ASME rated for the above temperature and resulting pressure.

3.2 AUTOMATIC CHECKOUT EQUIPMENT

Automatic Checkout and Readiness Equipment (ACRE) was used to test the printed-circuit cards and breadboard assemblies at the various stages of manufacture. ACRE was originally designed for, and has accomplished checkout of, the Polaris A1 and A2 missiles. The ACRE system, as used in the study application, consisted of two units: (1) the control computer, and (2) the test station (see Fig. 3-6).

3.2.1 *Control Computer*

The control computer is a special-purpose, externally-programmed, digital comparator. It is programmed by a pre-punched, eight-channel paper tape containing the sequence of operation and test limits.

The computer performs the following functions

- Controls the source and destination of all information transfers in the test station, and commands the test station to supply stimuli to the article under test

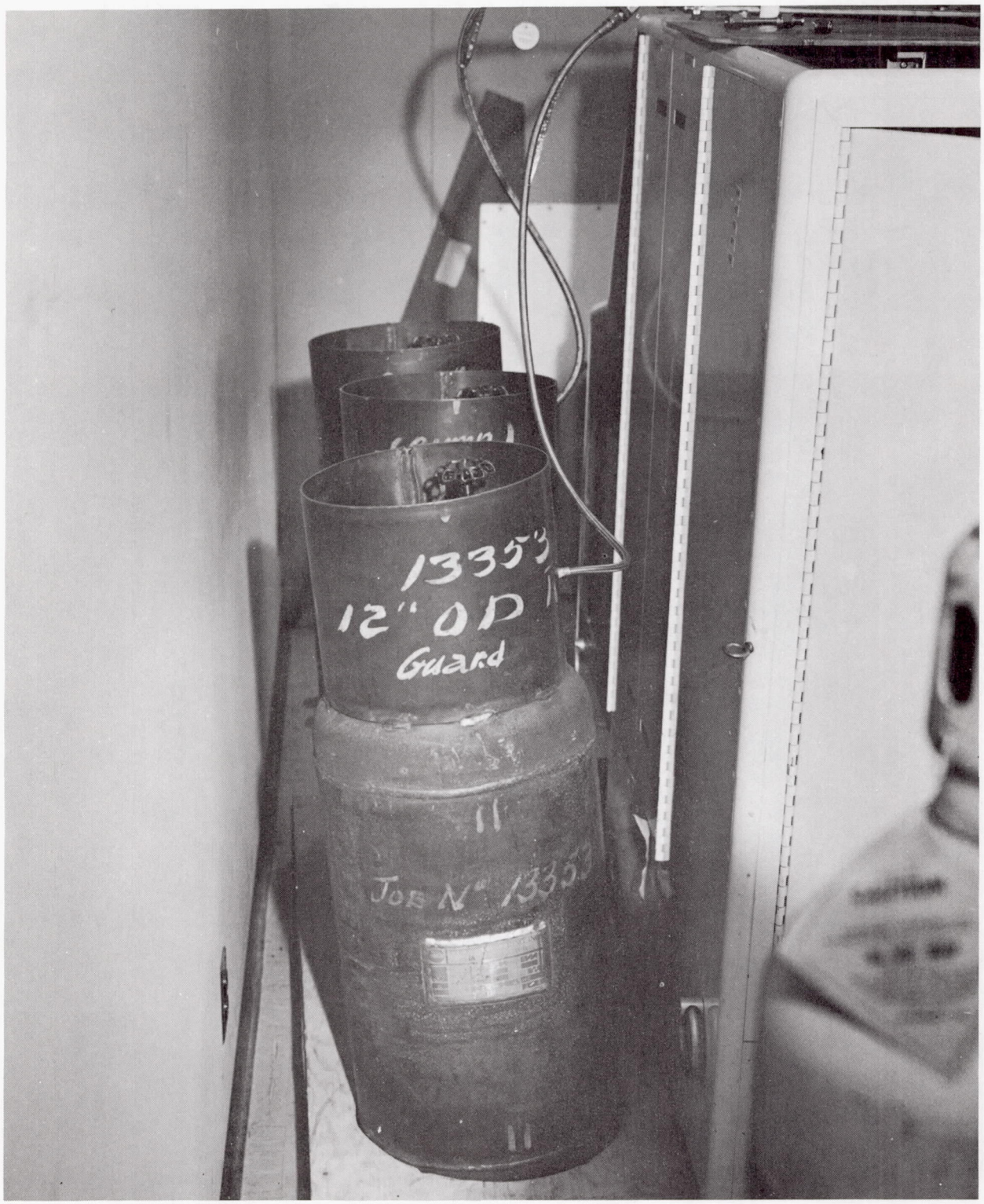


Fig. 3-5 Sterile-Air and Sterile-Nitrogen Flasks

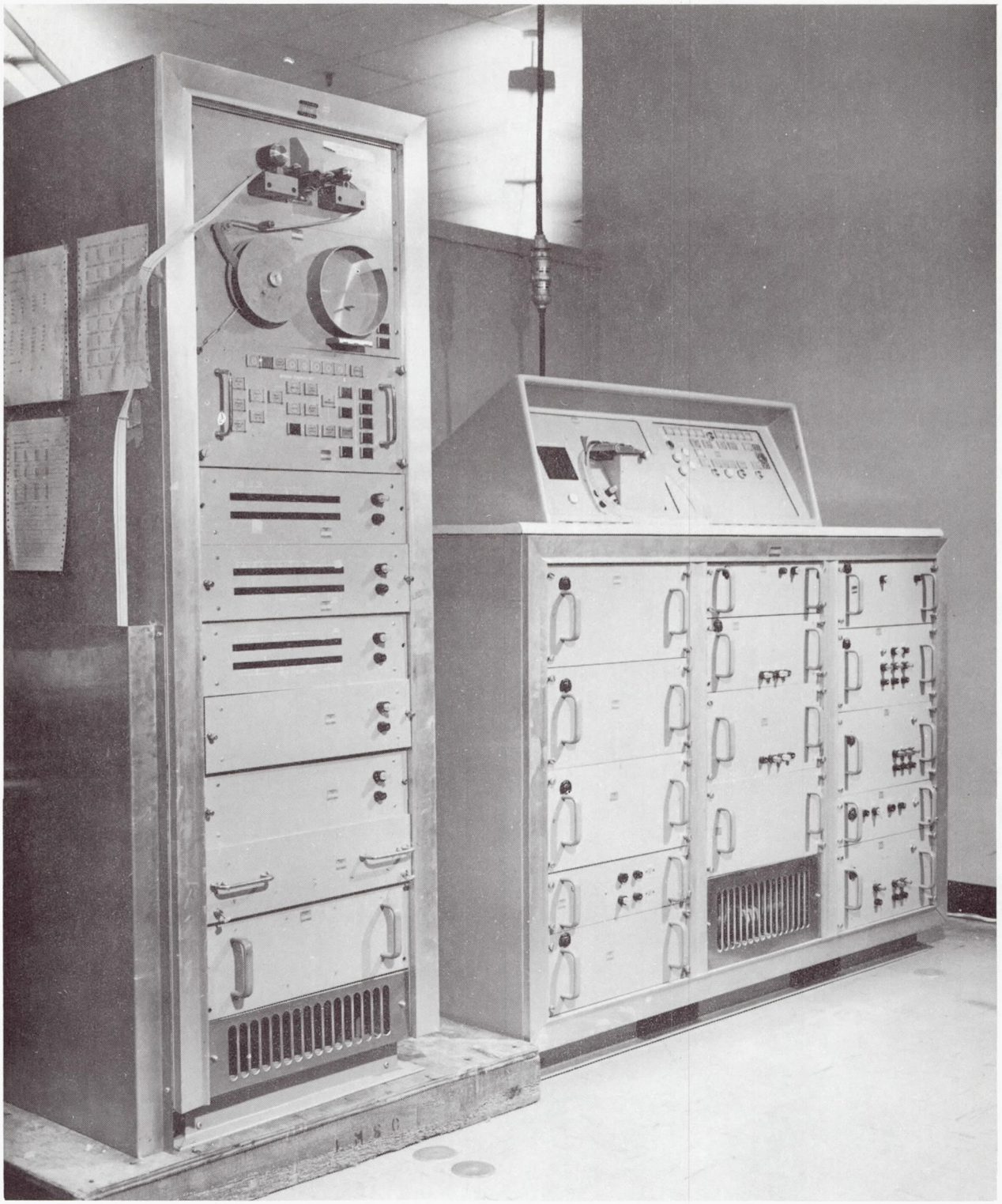


Fig. 3-6 ACRE Checkout Equipment – Control Computer (left) and Test Station (right)

- Controls the types of operations that are performed at each programmed step
- Receives signals returned from the system under test in response to the stimuli applied
- Performs the required computations on the response and makes a go/no-go decision
- Sends the results of these computations to a flexowriter in decimal form for printout

The control computer performs the above functions through the use of the following subsystems:

- Voltage-to-digital converter
- Frequency or time-to-digital converter
- Photo-electric punch tape reader
- Control panel with indicator lights and relays for switching functions
- Digital switching logic

3.2.2 ACRE Test Station

The ACRE test station contains the required equipment to supply the stimuli to the unit under test, and to route the response back to the control computer.

The following list of test-station subsystems includes only the equipment used for checkout in the Experimental Study of Sterile Assembly Techniques:

- AC/DC dual channel amplifier
- Matrix switching
- Programmable power supply
- Punched card reader
- Maintenance unit

The programmable power supply, through the matrix switching, supplied the dc excitation (+18 volts) to the printed-circuit card. All response signals from the card were routed back through the matrix switching to the ac/dc dual-channel amplifier. An

analog line carried all responses from the dual-channel amplifier to the control computer. The card reader was used to cause the flexowriter to print column headings prior to data printout.

The maintenance unit manually simulates the control functions from the control computer. This facilitates troubleshooting and programming.

The ac/dc dual-channel amplifier is used for the voltage ranging of the system. The amplifier delivers a conditioned signal to the control computer in the range of 0 to 8v. The amplifier accepts an input of from 125 mv to 256v.

Self testing is a built-in feature of this system. A standard cell of 1.01934 volts is included as a test point in the test station. This value is applied to points throughout the system to check for any drift prior to applying stimuli to the unit under test.

3.3 ATMOSPHERE MONITORING EQUIPMENT (LIRA 300 GAS ANALYZER)

This unit is a nondispersive infrared gas analyzer employing two LIRA 300 units specifically designed for ethylene oxide gas concentration and water vapor content determination (see Fig. 3-7). The analyzer was developed by Mine Safety Appliance Company (MSA) as a complete system. It contains five inlet ports and two exhaust ports plus interconnecting plumbing to the LIRA analyzers. Flowmeters and control valves are provided to regulate the gas flow to each unit. Specifications of the unit are as follows:

- Dimensions: 28 in. × 25 in. × 37 in.
- Flow Control: 0–3 liters per minute
- Inlets: 5 (zero, span, vaporized sample, humidified sample, pumped sample)
- Outlets: 2 exhausts and 2 relief exhausts
- Range:
  - Ethylene Oxide (0-30 percent by volume)
  - Water Vapor (0-1 percent by volume)

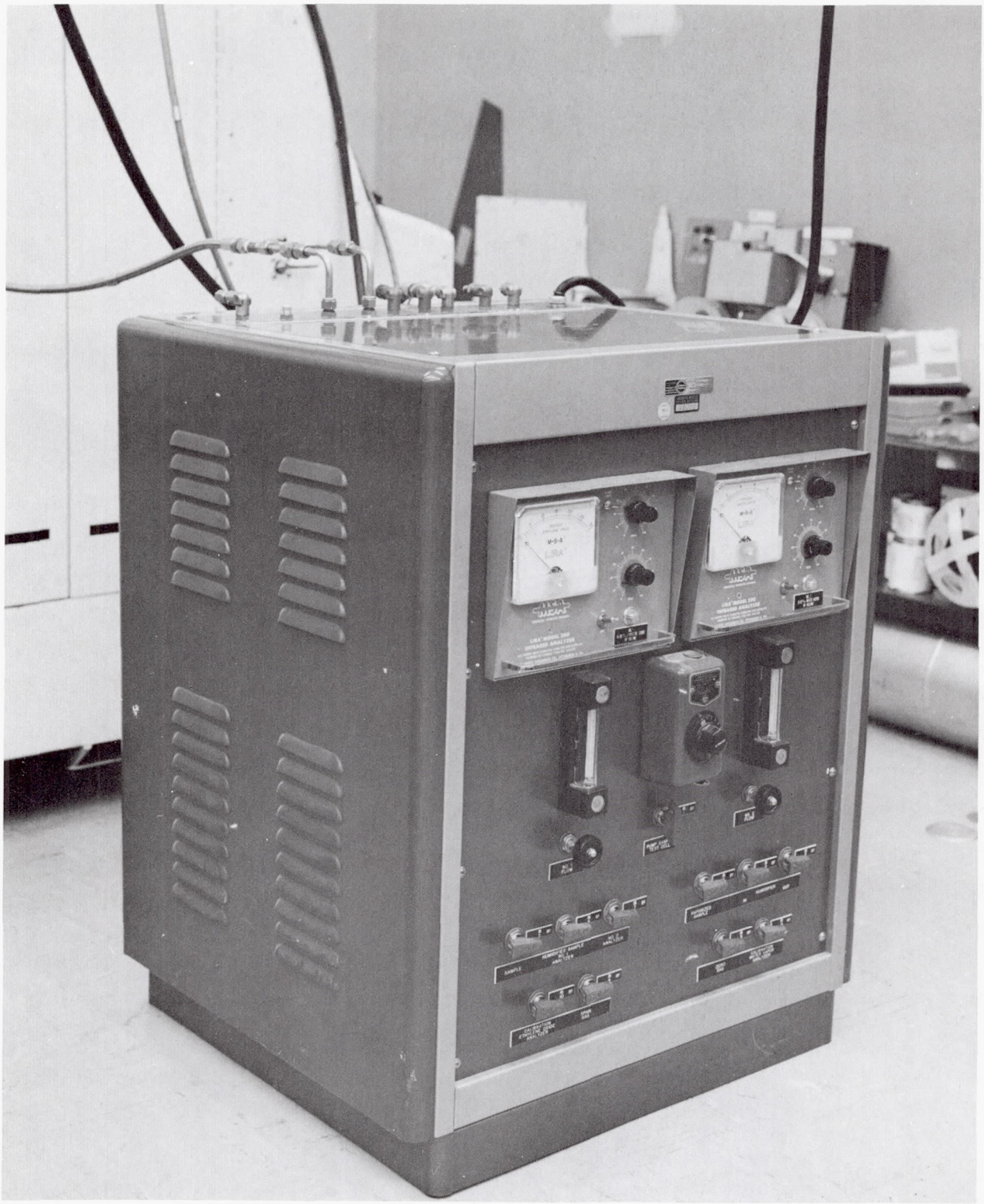


Fig. 3-7 Mine Safety Appliance Co. (MSA)
LIRA 300 Gas Analyzer

- Special features include:
  - Heated internal plumbing
  - Built-in calibration unit for water vapor analyzer

3.4 PROBLEMS ENCOUNTERED AND SOLUTIONS

3.4.1 Sterile Air and Nitrogen Flasks

Following their initial charge, the ASME coded flasks from Cole Engineering Company were placed in an oven and heated to 175° C for 24 hours. After this exposure, it was found that the flasks had bled down from their initial pressure of 100 psig to about 10 psig. A bubble check of the flasks was made, and it was found that the safety valves were leaking at only 10 lb of internal pressure. Cole Engineering Co. was immediately contacted, and Cole, which in turn contacted the valve supplier, Bailey Valve Co., suggested that valves be replaced with ones of the same rating having a teflon seat. This was accomplished and the flasks again charged. It was decided to heat to a lower temperature, 150° C, to reduce the chances of valve leakage. At this temperature, pressure in the flasks increased from 100 psig to 182 psig, well below the safety valve setting of 250 psig. The tanks were removed from the oven after a 25-hour sterilization period, and after cooling, tank pressure was 90 psig. Operation in this mode proved satisfactory for the remainder of the program.

3.4.2 Sterilization Cart

Casualties in the ETO supply system of the sterilization cart resulted in the decision to connect the sterilant gas supply directly to the main glove box. These casualties were, for the most part, restrictions in the ETO piping of the cart. Some of these restrictions were caused by poor design, and others were due to the long period since the equipment had last been operated.

The first failure occurred when the low temperature cut-out solenoid, which is designed to cut off the flow of ETO in the event that the evaporator effluent has not been vaporized, was found to be operating intermittently. The valve was disassembled and the disc and stem removed, effectively taking the valve out of the system.

The humidifier assembly on the ethylene oxide supply line of the gas cart was found to be clogged with a tar-like substance, probably ethylene glycol; the humidifier assembly was disassembled and cleaned. However, flow was again restricted after a short period of operation. At this point, the two-stage Victor reducing valve in the ethylene oxide supply line was disassembled and found to be clean. One stage of the valve was removed in order to reduce the valve's restriction to flow. The valve was then reassembled and reinstalled in the system. ETO flow was again attempted, and flow persisted for a few minutes and then essentially stopped.

At this time the ETO flask was disconnected from the sterilant gas generator and connected directly to the main glove box. Attachment was made at the pressure-gage connection to the box, and flow was controlled by a stop valve in this line. Flow was initially adequate using this system; however, it ceased after a short period. The flexible hose connection to the ethylene oxide supply flask was then disconnected, and the fitting which connects directly to the flask was found to contain a fine wire mesh screen designed to prevent any contaminents in the flask from entering the system. This screen was clogged with a white powder. The screen was drilled out and the bottle reconnected directly to the main glove box. As this system provided excellent flow control and was the utmost in simplicity, it was decided to leave the supply connected directly to the main glove box. In order to provide a sterilant gas connection to the gas generator system, a "T" connection was placed in the gage line to the main glove box. A hose was connected between this point and the sterile air supply line of the sterilization cart so as to permit the entire cart to be flooded with ETO for sterilization.

3.4.3 MSA LIRA 300 Infrared Analyzer

The MSA LIRA Infrared Analyzer in its designed configuration samples the effluent gas from the discharge side of the vacuum pump. The unit contains two cells, one reading water vapor content, the other ethylene oxide percent by volume. The MSA water vapor cell throughout the program gave an indication of high water vapor content

in the effluent gas. Readings yielded relative humidity values from 50 to 70 percent. The first-sterilization skips caused these readings to be questioned, and it was determined that the water vapor cell of the LIRA was not reading accurately, probably because of interferences from the ethylene oxide in the effluent stream. For the remainder of the program, a wet and dry-bulb humidity indicator was used as the primary method of relative humidity determination. The relative humidity control approach is discussed in more detail in Section 15 of this report.

Ethylene oxide concentration determination by the other LIRA cell was complicated by several factors. Investigation showed that flow of one liter per minute was required to obtain a steady reading from the cell. This was easily obtained with the MSA connected to the discharge side of the vacuum pump. The pump, however, unless the sterilization cart discharge valve was fully open, would suck air from the laboratory and lower the ethylene oxide concentration in the gas delivered to the LIRA cell. This was partially solved by connecting the MSA directly to the MGB and increasing pressure in the box to a 3 in. $H_2 0$ maximum. A second major disadvantage of the LIRA was the calibration procedures. A zero gage reading was first obtained with nitrogen flowing through the cell, and the gage was then spanned using the gas mixture directly from the flask. Proper effluent ETO concentration is then indicated when this span reading is reached. The reading is, therefore, a relative relationship between supply and effluent gases and is meaningful only if the supply gas has been accurately mixed by the supplier.

The problem is further complicated by the fact that the ethylene oxide and Freon 12 have different boiling points and molecular weights. Small vapor samples drawn from the flask for calibration purposes might therefore, be rich in either constituent. This made only a rough calibration possible and subordinated the MSA LIRA's ethylene oxide cell to a rough rather than absolute indicator of ethylene oxide concentration in the MGB. A discussion of the problems associated with the measurement of ethylene oxide concentration in the gas mixture is contained in Section 15. The laboratory techniques of chromatography and spectroscopy were used to determine that the content

of the gas in the cylinders supplied was within specification. These techniques were also used to measure the contents of the MGB. The best method of maintaining the concentration of ethylene oxide in the main glove box was to thoroughly purge at frequent intervals each week, and to alternately purge and evacuate the entry lock with ETO when passing items into the MGB.

3.5 OPERATIONAL COMMENTS

A simplified diagram of the sterilization laboratory and schematic of interconnections between major units of equipment is shown in Fig. 3-8.

3.5.1 Sterilization Cart and Glove Box System Operation

The entire glove box system was initially filled with the ethylene oxide/Freon 12 (ETO) gas mixture in order to sterilize all chambers and lines prior to commencement of actual test-item sterilization. The gas mixture was initially introduced into the baffled plenum chamber upon which the working section of the main glove box rests. The baffles distribute the ETO throughout the chamber and cause the ETO to rise slowly in the glassed-in portion of the box. A suction was taken with the sterilization cart vacuum pump from the top of the box as the air was displaced slowly by the incoming ETO. Suction was continued until the MSA LIRA infrared cell indicated that the effluent from the box gave the same reading as a calibration reading made using the incoming gas. Midway in this procedure, the ETO supply was connected directly from the flask to the MGB (main glove box.) Using this technique, the ETO would often not vaporize in the line between the flask and the MGB. The liquid was delivered to a metal pan in the MGB where it was allowed to vaporize. No problems were experienced using this technique.

When the main glove box was full of ETO, suction was shifted to the SGB (small glove box). The doors between the interchange lock, the MGB, and the SGB were opened, and the ETO was allowed to flow into these chambers. Suction was shifted to the SGB; again flow was continued until the effluent indicated full ETO concentration. This procedure was repeated to fill the entry lock. ETO was then allowed to back flow into the

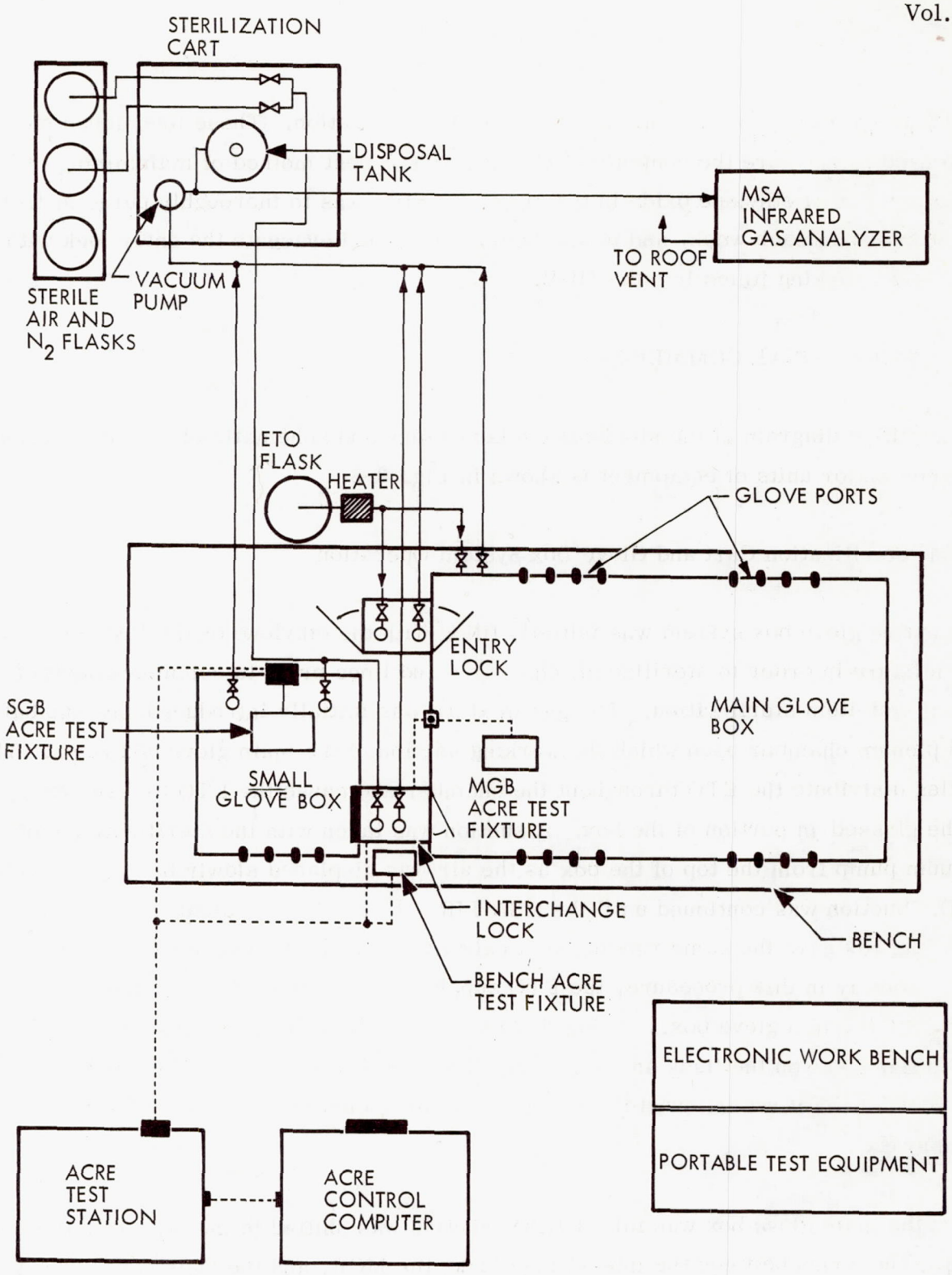


Fig. 3-8 Sterilization Laboratory Layout With Major System Interconnections

sterilization cart, which was vented at all possible points until the ETO was detected. This procedure was used to sterilize the cart whenever a fresh sterile-nitrogen or sterile-air flask was connected to the cart. The glove box system and the cart were allowed to soak for 24 hours before the first test samples were introduced into MGB.

3.5.2 Purging Operations

ETO was purged from the SGB using sterile air prior to bottling operations. Two flasks were used for the initial purge. The ETO sterilization soak of the SGB, followed by a sterile air purge, was repeated when it was discovered that non-sterile test specimens had been bottled in the SGB. The interchange lock was soaked and purged concurrently with SGB. Midway in the program, it was found that the bottling operation could be successfully accomplished in the MGB if the time during which the jar lids were opened was minimized. This technique was often used when bottling special test specimens for contamination analysis.

The entry lock was used to pass test items and equipment into and from the MGB. On entry, the items were placed in the lock and the outer door shut. A vacuum of 30 in. of water was then pulled on the lock, and ETO directly from the flask on service was then charged into the lock to a positive pressure of 0.5 in. This operation was repeated at least five times. The inner door, on the MGB end of the lock, was then opened and the material passed into the MGB. To take items from the MGB to the laboratory, the procedure was reversed using air to purge the lock after drawing the vacuum. The interchange lock was operated in a similar manner.

All chambers in the glove box system were maintained at a positive pressure throughout the program. None of the chambers was absolutely leak tight, but in all cases, the pressures remained positive on weekends and vacation periods. The MGB was generally charged to at least 1 in. of H_2O each morning. After work in the MGB which involved hand or dip soldering, or several entries or exits from the box, the chamber was purged with ETO.

Section 4
BIOLOGICAL MATERIALS AND METHODS

4.1 TEST ORGANISM

Bacillus globigii was used as the test organism. The spore strain used was received (deposited on sterile cloth patches) from Dr. Charles Phillips, Fort Detrick, Maryland. When grown on trypticase soy agar, intense orange pigmentation was formed by all colonies. This characteristic color, combined with the typical colony shape and texture, enabled easy recognition of the test organism and of extraneous contaminants.

4.2 SPORE PREPARATION

A spore preparation of Bacillus globigii was prepared by the following method: Two-liter Erlenmeyer flasks containing 400 ml of trypticase soy broth were inoculated with 10^{10} Bacillus globigii spores. After seven days incubation at 37° C with constant shaking on a New Brunswick Model G10 shaker, the cells were harvested in a refrigerated contrifuge, resuspended in a portion of the original growth medium, and pasteurized by heating at 80° C for 30 minutes. This spore preparation was stored at 5°C in a Screw Cap Erlenmeyer flask. This initial count was 10^{10} spores per ml.

This spore preparation was used in all testing procedures during the contract period. The number of viable spores in the preparation was determined at frequent intervals. The spore numbers were remarkably constant as shown in Fig. 4-1. The slight increase shown in Fig. 4-1 might be a reflection of incomplete mixing before removal of material for spore inoculations.

It is to be noted that no attempt was made to free the spores of cell debris. The presence of extraneous organic material should give a maximum challenge condition for the sterilizing agent. Also, the presence of spores in occluded cell debris is most representative of conditions to be encountered under actual working conditions.

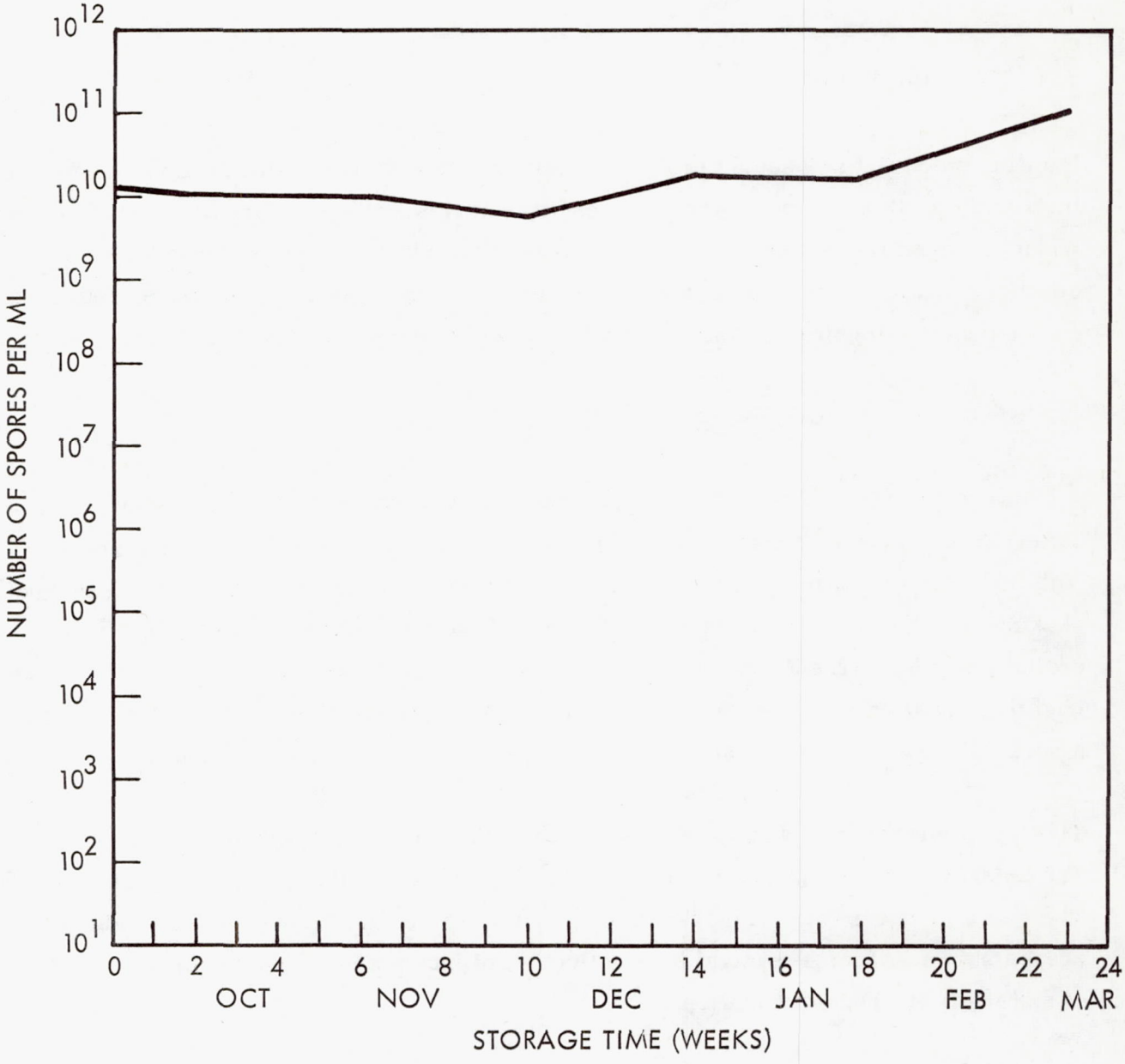


Fig. 4-1 Viable Spore Concentration as a Function of Time

4.3 INOCULATION OF COMPONENTS

The individual test items were heat sterilized for 24 hr at 13° C in a dry air oven. Spores of Bacillus globigii were deposited on individual electronic components by dipping into an aqueous suspension containing approximately 10^8 spores per ml. The components were then removed with sterile hemostats and suspended in a dry air oven at 45°C for 15 min. These components were then packaged in suitable sterile containers until utilized in the assembly operations.

Sample components were tested to determine the level of spore deposition achieved. The component was agitated in 100 ml of sterile water, and the number of organisms present determined by plating the appropriate dilutions on trypticase soy agar.

4.4 TESTING FOR STERILITY

Trypticase soy broth was heat sterilized in screw-capped bottles (125 ml in 8 oz bottles), or tubes (10 ml in 20 by 125 mm tubes), and then placed in the assembly box before sterilization with ethylene oxide. Each heat sterilization cycle was monitored with a spore strip of Bacillus stearothermophilus (American Sterilizer).

When a test sample was assembled, it was immediately placed in trypticase soy broth. The sample was then placed on the gyratory shaker at 37° C for seven days. At the end of seven days the sample was removed, examined visually, and a wet preparation examined microscopically. A 0.1 ml aliquot was plated on trypticase soy agar and another 0.1 ml aliquot added to 10 ml of trypticase soy broth. After incubation at 37°C for 48 hours, these were examined for growth.

After the seven-day incubation at 37°C, the culture medium containing the test sample was inoculated with 100 Bacillus globigii spores and again incubated at 37°C with shaking. Three examinations for growth – turbidimetrically, microscopically, and by trypticase soy agar plating – were performed at the end of this time. Absence of growth indicated toxicity of the component.

4.5 SPORE STRIP PREPARATION

Following the first incidence of nonsterile test items, spore strips were placed in the ETO atmosphere along with all items being sterilized (See Fig. 4-2). These were prepared from 1 cm by 7 cm Whatman No. 1 filter paper strips, having a hole with a string loop at one end, impregnated with 10^6 spores of Bacillus globigi, and air dried. In use, the strips were suspended by the string in the MGB. At the end of the sterilization period, and immediately preceding any assembly operations, the spore strip was placed in one of the screw cap bottles containing culture media and tested bacteriologically by the standard procedure. Spores were also deposited in the same manner on glass plates and used in parallel with the paper spore strip. The glass strip is also shown in Fig. 4-2.

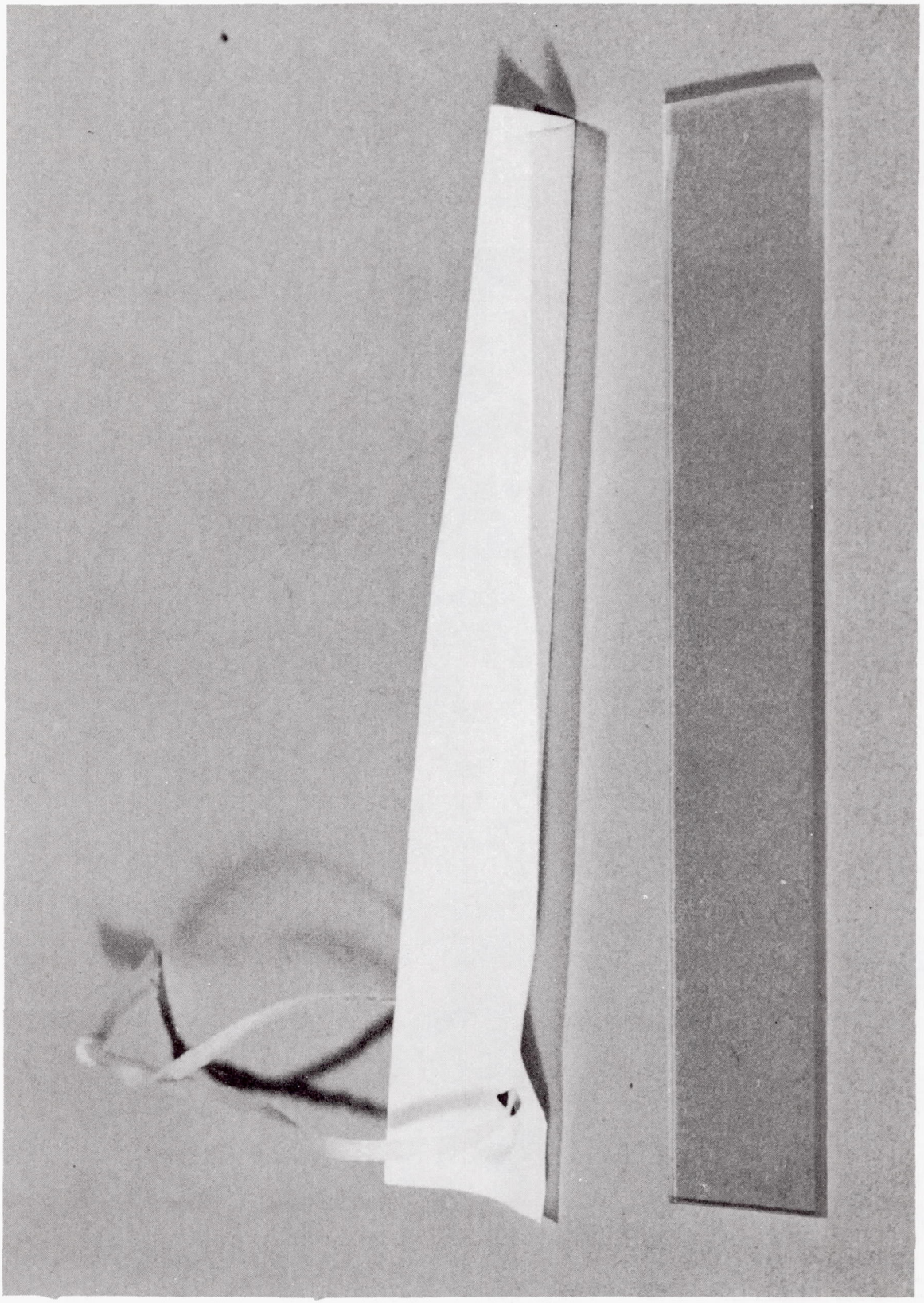


Fig. 4-2 Spore Strips – Paper (top) and Glass (bottom)

Section 5
TOXICITY TESTS

5.1 APPROACH

The object of the bacteriological testing in this program was to determine sterility – the absence of all viable organisms. Growth conditions, when a sterile assay is conducted, should allow one such organism to be detected. For this reason toxicity of culture media was determined by adding viable spores to each test culture. Early in the program, toxicity was tested by adding 10^6 spores to each solution. At JPL's suggestion, this inoculum was reduced to 10^2 spores. Toxicity tests were performed for the majority of the program using this reduced number of spores.

Tests of each circuit component, the printed-circuit card, and the solder were first conducted. Each item was pretreated by heating at 135° C for 24 hours, and then exposed to ethylene oxide/Freon sterilant gas at room temperature for 24 hours. The components were then placed in trypticase soy broth at 37° C for one week. The nutrient was then inoculated with viable spores and checked for growth. If growth occurred, the component was considered to be nontoxic.

Throughout the program, each jar of nutrient containing test items which did not produce growth after seven days of incubation was inoculated with viable spores. If growth did not occur after a subsequent seven-day incubation, the nutrient was considered to be toxic.

5.2 PROBLEMS ENCOUNTERED AND SOLUTIONS

5.2.1 Solder Toxicity

The initial tests of the rosin-core solder indicated that the solder partially inhibited growth of the test organism. If the solder was first melted using a soldering iron, the inhibitory properties of the solder were removed. This can be explained by volatilization and/or decomposition of the organic rosin at a high temperature which occurs in the normal soldering process. Toxicity noticed at various stages in the program was not believed to have been caused by the solder.

5.2.2 Printed-Circuit-Card Toxicity

Printed-circuit cards with uncoated copper discolored the trypticase soy nutrient. As some toxicity was detected in the early tests using an inoculum of 10^6 spores, a special test was run to establish the toxicity of an uncoated printed-circuit card.

The circuit board was placed in 100 ml of trypticase soy broth and shaken for seven days. At this time the broth had a blackish-green color. A 10 ml aliquot was centrifuged at high speed and another 10 ml aliquot filtered through a 0.3 micron Gelman filter. In both tests there were no indications of any solid material. Therefore, the discoloration was produced by materials in true solution. Ten ml portions of this blackish-green solution were placed in sterilized-screw-capped test tubes and inoculated with Bacillus globigii spores, incubated at 37° C for seven days, and then examined for viable organisms, as shown in Table 5-1.

Table 5-1

PRINTED-CIRCUIT-CARD TOXICITY TEST

Original Number Spores/Culture Tube	0.1 ml Plated After 7 Days	Total Tube Contents Filtered 0.3 μ Gelman	Total Viable Organism After 7 Days
10^9	300	–	30,000
10^8	300	–	30,000
10^7	18	–	1,800
10^6	1	–	100
10^5	0	10	10
10^4	0	0	0
10^3	0	0	0
10^2	0	–	–
10	0	–	–
0	0	–	–

Table 5-1 shows that this solution inhibited growth and was also bacteriocidal.

Tests conducted with solder-coated printed-circuit cards resulted in much less toxicity, and there were several instances where uncoated copper cards were contaminated. Laboratory observations indicated that growth most often occurred in the first 24 to 48 hours, probably before a serious toxic concentration had developed in the growth media. There is, therefore, strong evidence that those test jars which showed no growth during the sterility test period and were toxic after seven days, contained sterile test items.

5.3 OPERATIONAL COMMENTS

Using the standard incubation procedures, it was determined that eight Bacillus globigii spores, when placed in 15 ml trypticase soy broth, gave excellent growth in 22 hours. When eight spores were introduced into 100 ml of medium in screw-cap bottles and incubated on the shaker at 37°C, 3×10^9 organisms were present after 22 hours. Therefore, rapid multiplication of Bacillus globigii spores occurs under the standard test conditions.

5.4 RESULTS

Table 5-2 is a summary of items tested for toxicity during the toxicity and process test phases. IRC resistors and Vitamin Q capacitors were not toxic. The printed-circuit boards with copper tracks usually were toxic after a seven-day test period. However, contaminated assemblies containing circuit boards have been obtained as discussed in subsection 5.2.2 above. The transistors were toxic when placed in 15 ml of broth, but supported growth when placed in 100 ml.

These results would indicate that the ratio of sample-to-culture volume, and the time of exposure of the sample to the culture medium, is of importance in determining the degree of toxicity of each component.

The number of toxic components encountered indicates the difficulty of testing complex equipment for sterility. For many test samples, removal of the test material after a short period of agitation with the culture medium might be desirable.

Table 5-2

COMPONENT TOXICITY

Type	Number Tested	Toxic 10^2	Toxic 10^6
IRC resistor	50	0	–
Vitamin Q capacitor	160	0	–
Alpha solder	3	–	(Some toxicity)
Alpha solder burned	1	–	0
Transistor (3 wire)	6	2 (15 ml, not in 100)	0
Nut-bolt assembly	10	–	10
Small black diode	4	0	–
Induction coil	7	0	–
Circuit boards	3	–	3
Gold plated	1	–	1 (smaller degree of toxicity)
Solder dipped	1	–	1 (less toxic than undipped)
8 strips in jar	1	1	–
7 strips in jar	1	1	–
5 strips in jar	1	1	–
Metal plate to printed-circuit card	5	5	–
Metal plate to metal plate	5	3	–

Section 6
STERILIZATION TIME-PERIOD DETERMINATION

6.1 APPROACH

The first operational task in the program was to establish the time required for sterilization under the conditions existing in the main glove box (MGB). Small resistors, which had been inoculated with B. globigii spores, were placed in the MGB for varying time periods; and then they were bottled in the small glove box (SGB) in a sterile air atmosphere. This was followed by incubation at the LMSC biology laboratory in Palo Alto. Specimens were passed into the MGB and remained there for 48 hours, 28 hours, 24 hours, 15 hours, and 4 hours. Ten items were used for each sterilization time period determination.

The resistors were placed on cheese cloth within the box. Five of the 10 resistors in each phase were turned at approximately twelve hour intervals. The other five were not turned. This was done to ascertain if the cheese cloth permitted enough circulation of sterilant gas to all surfaces of each component.

Later in the program, sterilization skips made it necessary to conduct an additional sterilization time period determination. Inoculated capacitors rather than resistors were used during this test phase. Tests were run for 48 and 72 hours. The capacitors were hung on nylon cords strung parallel to the longitudinal axis of the MGB. Knots in the cords kept the capacitors separated.

6.2 PROBLEMS ENCOUNTERED AND SOLUTIONS

No major problems were encountered in the implementation of either sterilization time period determination conducted. In the course of the study, it was shown that the

resistors were one of the easiest components in the test circuit to sterilize. It was unfortunate that this item was the arbitrary selection for use in determining the sterilization time period. Had the capacitors been used, the sterilization skip problem might have been uncovered during the early days of the program.

The capacitor test was run following installation of the nylon cord "clothesline" and the use of a small circulating fan to keep the MGB atmosphere in motion. Although the formal sterilization time period determination test using capacitors had encouraging results, sterilization skips continued to occur after this test was conducted.

6.3 OPERATIONAL COMMENTS

Turning the small resistors on the cheese cloth proved to be a problem. The resistor leads would tangle in the wide mesh of the cheese cloth, but with practice the operation was performed with ease.

The resistors, following their inoculation in the Palo Alto biology laboratory, were delivered to Sunnyvale in glass jars. One end of the resistors was inserted in a plastic foam disc at the bottom of the jar. This made handling much easier within the MGB and also prevented resistors from contacting each other and the side of the jar.

The capacitors were hung on the "clothesline" by forming one lead in the shape of a hook. The nylon line would act like a bow-string and flip off the hanging components unless care was taken during hanging and removing the capacitors from the line.

6.4 RESULTS

Table 6-1 shows the results of the first sterilization time period determination using resistors. Detailed results of this test are found in Appendix A, pages A-2 and A-3. After the results of this test, it was decided that a soak period of at least 48 hours would be adequate to insure sterilization.

Table 6-1

STERILIZATION TIME – RESISTORS

Phase	Number of Items	Results
48 hour	10 5 turned (A) 5 not turned (B)	All sterile
28 hour	10	All sterile
24 hour	10	All sterile
15 hour	10	2 - (A) not sterile 1 - (B) not sterile
4 hour	10	1 - (A) not sterile 1 - (B) not sterile

Table 6-2 shows the results of the sterilization time period determination using capacitors. These results indicated that the sterilization skip problem had been solved by hanging the components, the addition of forced circulation, and better control and indication of humidity in the MGB. With the above improvements in atmosphere control, it was believed that a sterilization time period of at least 72 hours would prove to be adequate. See Appendix A, page A-4, for detailed sterilization assay information.

Table 6-2

STERILIZATION TIME – CAPACITORS

Phase	Number of Items	Results
48 hours	10	All sterile
72 hours	10	All sterile

Section 7
HAND SOLDERING PROCESS TESTS

7.1 APPROACH

The process of hand soldering was first tested in the main glove box (MGB) in ETO by soldering a wire to a resistor. One resistor lead was clipped and then soldered at the point where the cut was made (Fig. 7-1). Prior to start of the test, all resistors to be used were measured accurately and their precise resistance value noted. The resistors were then separated into groups to be assembled on the bench and in the MGB. The MGB-assembled specimens were further divided into those which would be assembled and then passed into the small glove box and bottled for sterility test, and those which were to be removed from the glove box system and performance tested. The resistor sterility test items were passed into the small glove box where the solder joint was disassembled. This was accomplished using a pair of tin shears. The two wire leads were separated by cutting through the solder at their point of contact. The solder was then shaved from the wires to expose the portions of the wire which had previously been contaminated with organisms. All residue from the cutting operation was then placed in a jar of nutrient. A sheet of note paper was used to collect the bits of solder and wire from this operation.

A second performance evaluation of hand soldering was accomplished later in the program. Five solder joint samples were prepared in the MGB in ETO and five on the work bench in the normal laboratory air environment. The 10 joints were then photomicrographically analyzed by Lockheed's Material and Process Control Laboratory. The test samples consisted of wires soldered together in an "X" configuration. The joints were then sectioned and polished prior to photographing. Three of the samples manufactured in laboratory air and three of the samples manufactured in ETO were sectioned in the "X" direction, the other four in the "Y" direction (Fig. 7-2).

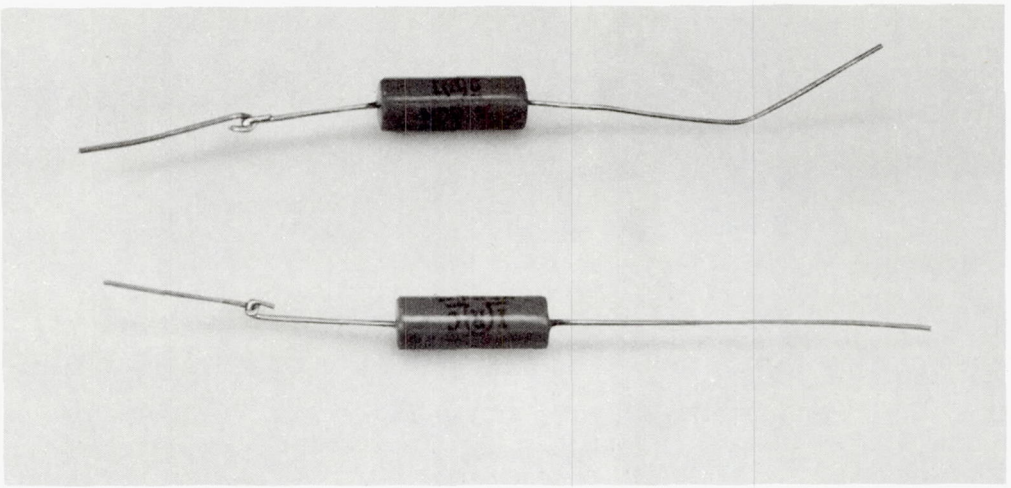


Fig. 7-1 Hand-Soldered Process Test Specimens

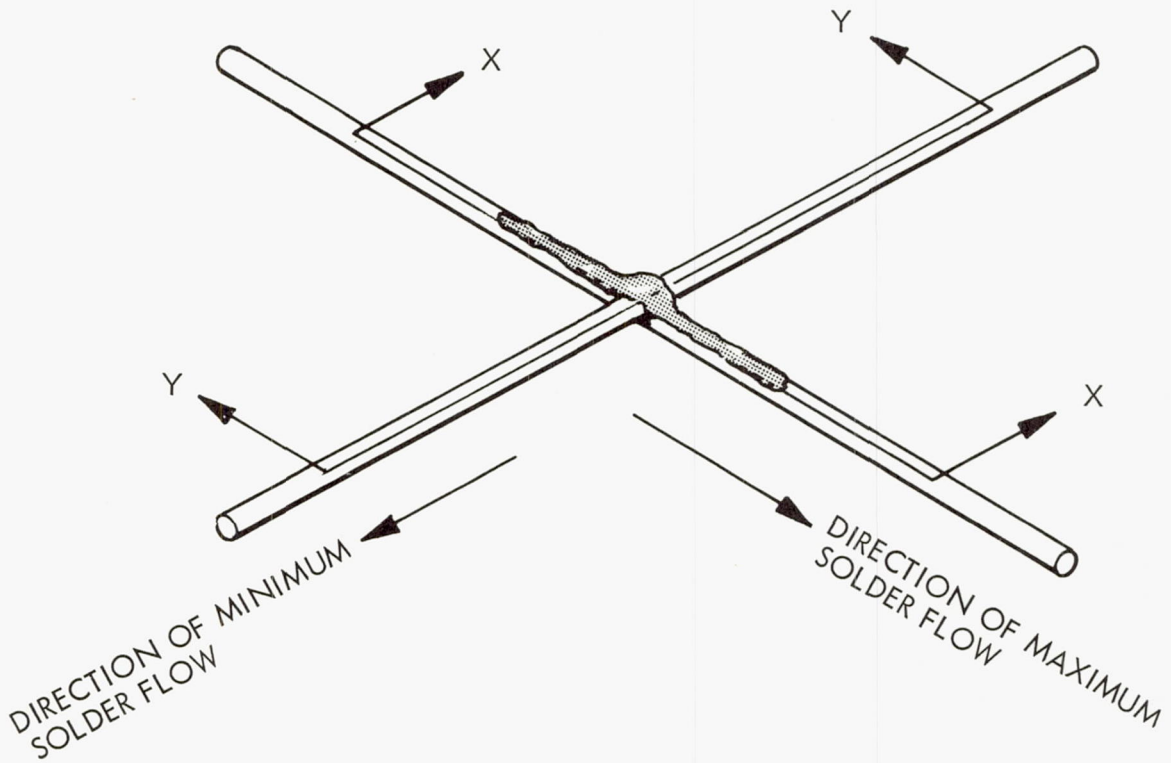


Fig. 7-2 Crossed-Wire, Hand-Soldered Process Item Showing Sectioning Method for Photomicrographic Analysis

7.2 PROBLEMS ENCOUNTERED AND SOLUTIONS

The major problem encountered in the hand soldering process test was one of soldering iron failure following operation in the MGB ETO atmosphere. The Hexacon brand soldering iron initially used in the test failed after approximately three minutes of operation in the glove box atmosphere. This is the standard Lockheed iron. A new iron of the same manufacture was then passed into the box and soldering was again attempted. This soldering iron also failed after about three minutes of operation. Both irons were then removed from the glove box system and disassembled. Both irons were found to have failed at the heating element which is composed of fine nichrome wire.

An Ungar brand iron was substituted for the Hexacon and was used for the remainder of the program. The Ungar has a replaceable ceramic sealed heating element and performs satisfactorily for longer periods of time. This iron, however, also burns out after use in the ETO atmosphere. The first element lasted for several days of intermittent operation. Its failure was first attributed to a break in the ceramic coating material. Six Ungar brand elements failed during the course of the contract. All failed while being used in ETO; none failed while being used on the bench, in sterile air, or in sterile nitrogen. The approximate average life of an element has been 12 hours; the shortest use time was about one hour.

Optical microscopic examination of the fused heating wires of several elements by the Lockheed Materials Sciences Laboratory personnel showed a dark crystalline coating rather loosely adhering to the wires. This was in contrast to the wires of soldering irons which had not been exposed to sterilizing gas and which showed only the normal thin, tightly bound layers of metal oxide.

Prior experience with thermal degradation of ethylene oxide and Freons on a solid surface directed suspicion to the Freon 12. This was confirmed by a short experiment.

Soldering irons were run in atmospheres of pure Freon 12 and ethylene oxide, each on an individual basis. Figure 7-3 is a schematic of the experimental apparatus used. Operation time in Freon 12 was 25 min to failure of the heating element. After 2 hours of continuous operation in ethylene oxide , the test soldering iron was still functioning.

7.3 OPERATIONAL COMMENTS

Some problems were experienced in handling the small wire which was soldered to the resistor lead. The wire was bent into a hook shape prior to soldering. On the first few tries, the wire to be soldered would spring off as it was hooked over the resistor lead. After some practice the operator was then able, by using his eyesight to compensate for his reduced sense of touch, to properly place the two wires and complete the soldering operation. No other operational problems were noted.

7.4 RESULTS

Electronic performance of the hand soldered resistors are shown in Table 7-1. All changes in resistance noted are considered to be minor and within the measurement tolerences of the instruments used.

The resistors which were checked for sterility were all uncontaminated with the exception of one of four resistors on which work was not performed, but which were in the MGB for the same period of time (Appendix A, page A-6). The contamination of this item might have resulted from several causes. One possible explanation is that this contamination was caused by cross contamination from dip soldering test items discussed in Section 8 and which were in the box at the same time. It is also possible that the contamination of the resistor might have been caused by failure of the gas to reach all surfaces. The four resistors not worked on were not handled in the glove box. They were not turned, but were allowed to rest at two points across a small

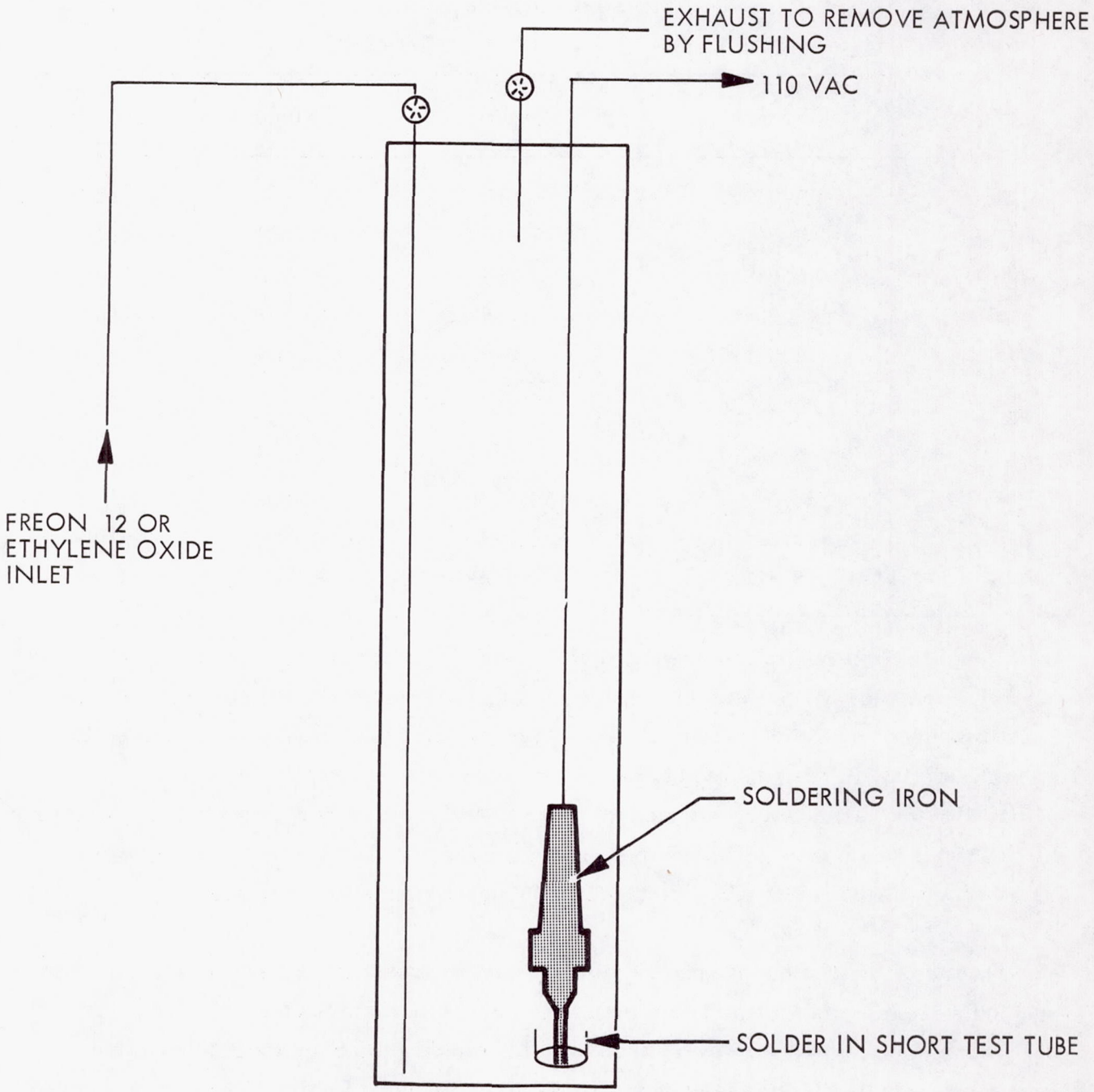


Fig. 7-3 Apparatus for Testing Effect of Atmosphere on Soldering Iron

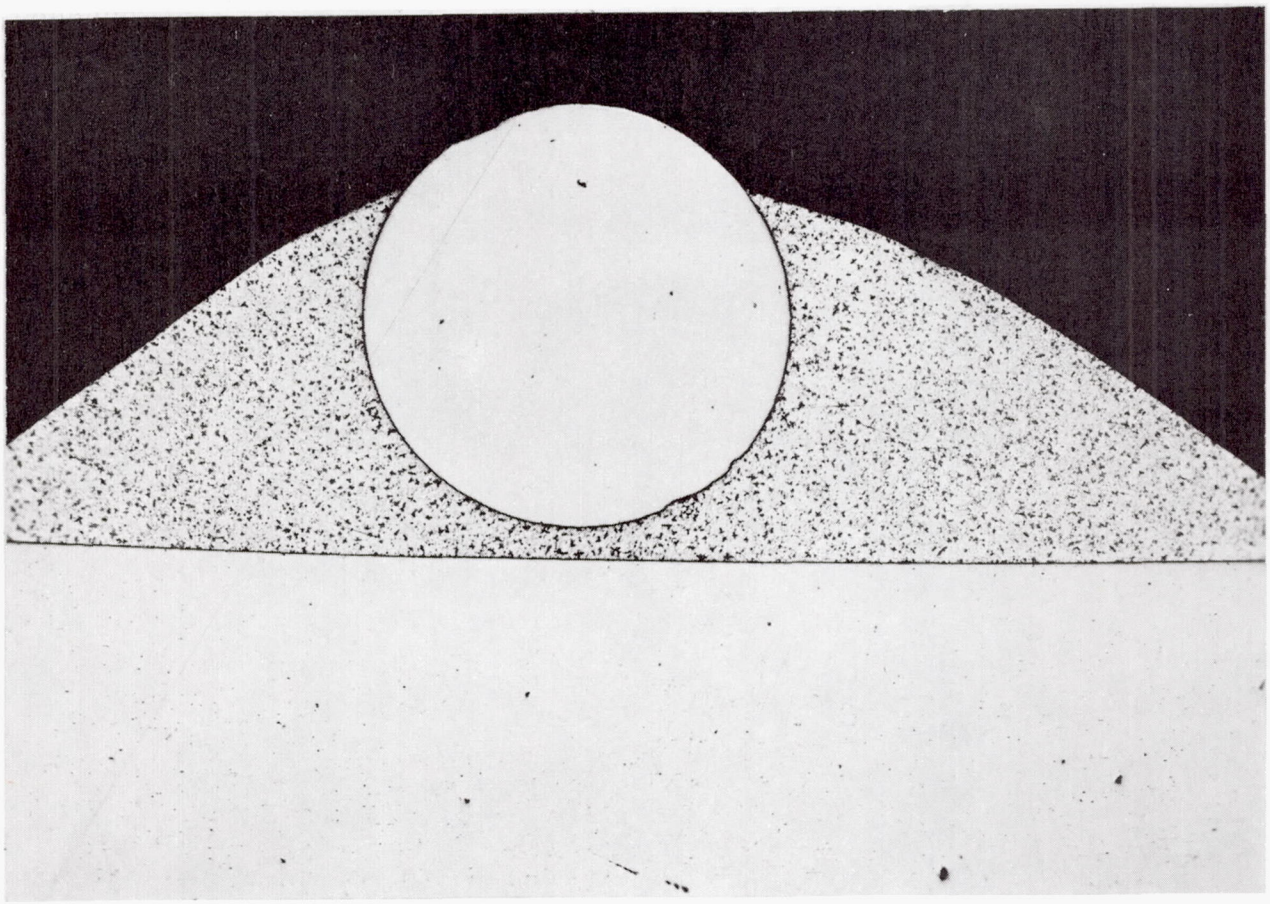
Table 7-1

RESISTOR MEASUREMENTS

Resistor	Value Before Assembly (ohm)	Value After Assembly (ohm)	Change (ohm)	Change (%)
P1	27.055	27.053	+0.002	0.007
P4	26.996	26.967	+0.001	0.004
P26	39.061	39.074	+0.013	0.033
P41	22.054k	22.100k	+0.046	0.200
P46	21.979k	21.989k	+0.010	0.045
P3	27.125	27.145	+0.020	0.070
P5	27.745	27.741	-0.004	0.015
P35	39.068	39.048	-0.020	0.051
P45	22.045k	22.031k	-0.014	0.064
P48	22.120k	22.078k	-0.042	0.190

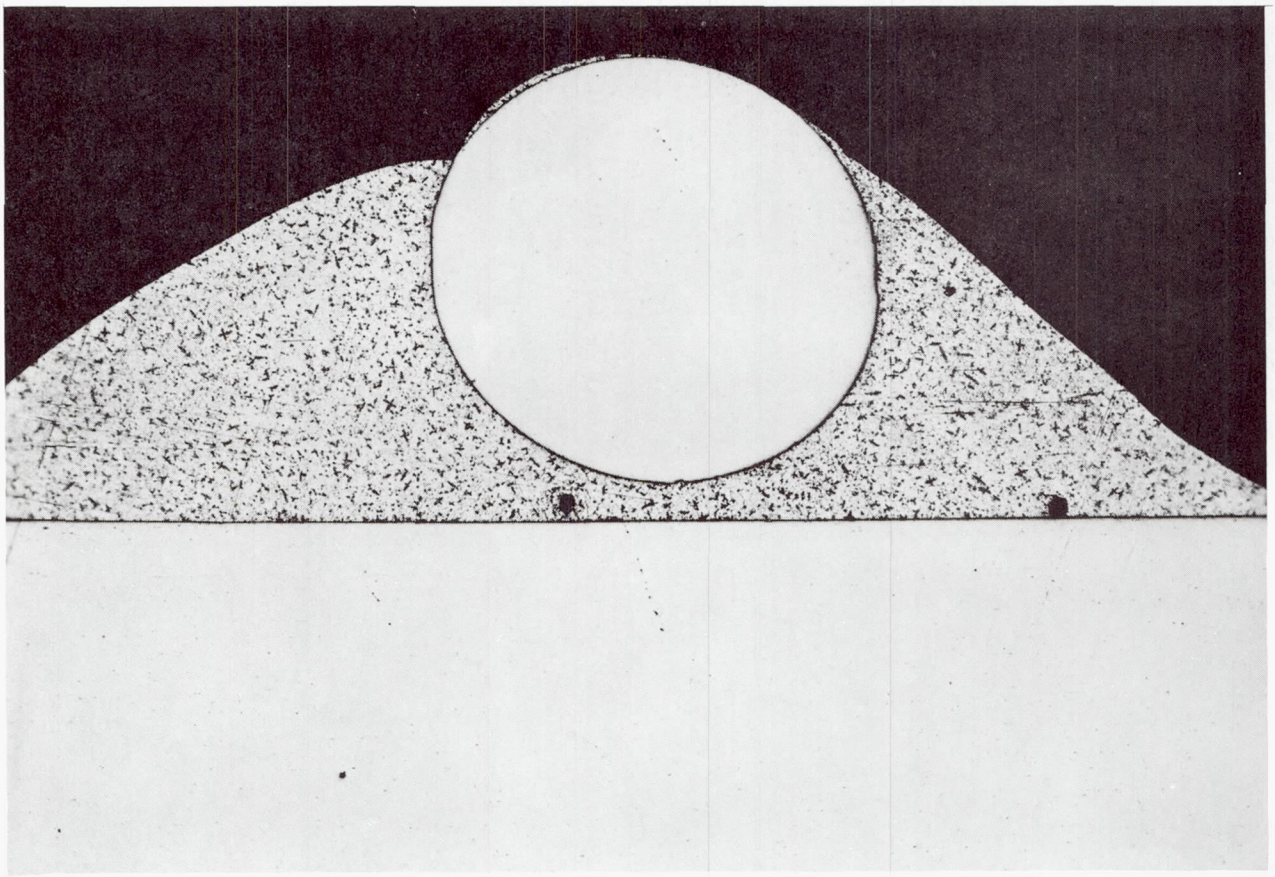
metal cup. It is possible that the gas did not reach the points at which the resistor leads contacted the cup. All subsequent components and assemblies being sterilized in the box were turned at approximately 12 hour intervals, or were hung on the nylon cords strung in the MGB. During the course of the study, few sterilization skips were associated with the resistors, which appeared to be one of the easiest components to sterilize using ETO. It is possible, however, that organisms deposited on this one non-sterile resistor were simply not killed by the ETO during the period of exposure.

The results of the photomicrographic solder joint analyses showed essentially no difference between the joints made in air and those made in the ethylene oxide/Freon 12. The report did mention, however, that the joints manufactured in the ETO seemed to have fewer small voids in the solder than did the units done in air. There was no noticeable difference in wetting action or structure of the solder. Four of the photomicrographics are presented in Fig. 7-4 through 7-7.



70×

Fig. 7-4 Photomicrographic: Sample No. 1, Made in Laboratory Air



70×

Fig. 7-5 Photomicrographic: Sample No. 5, Laboratory Air

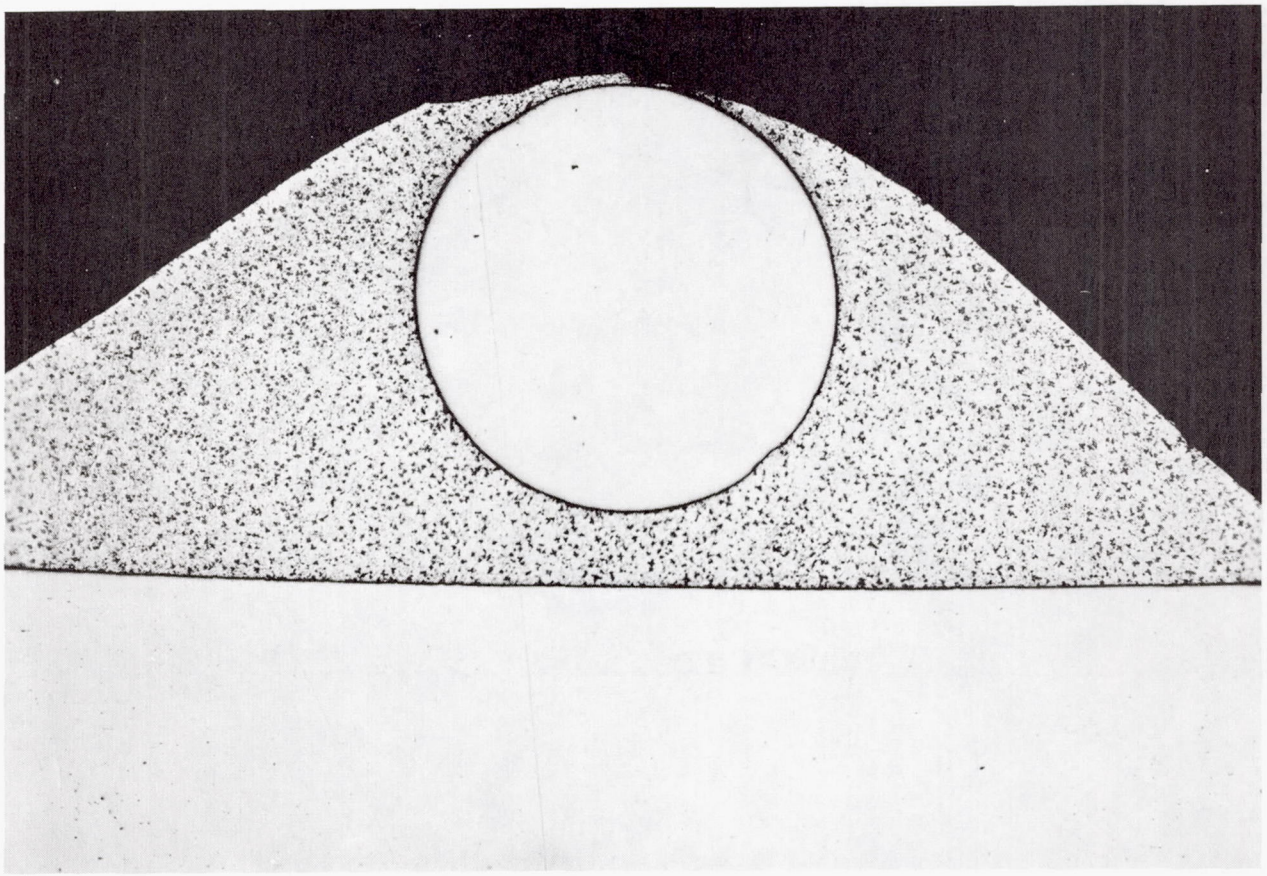


Fig. 7-6 Photomicrographic: Sample No. 6, ETO

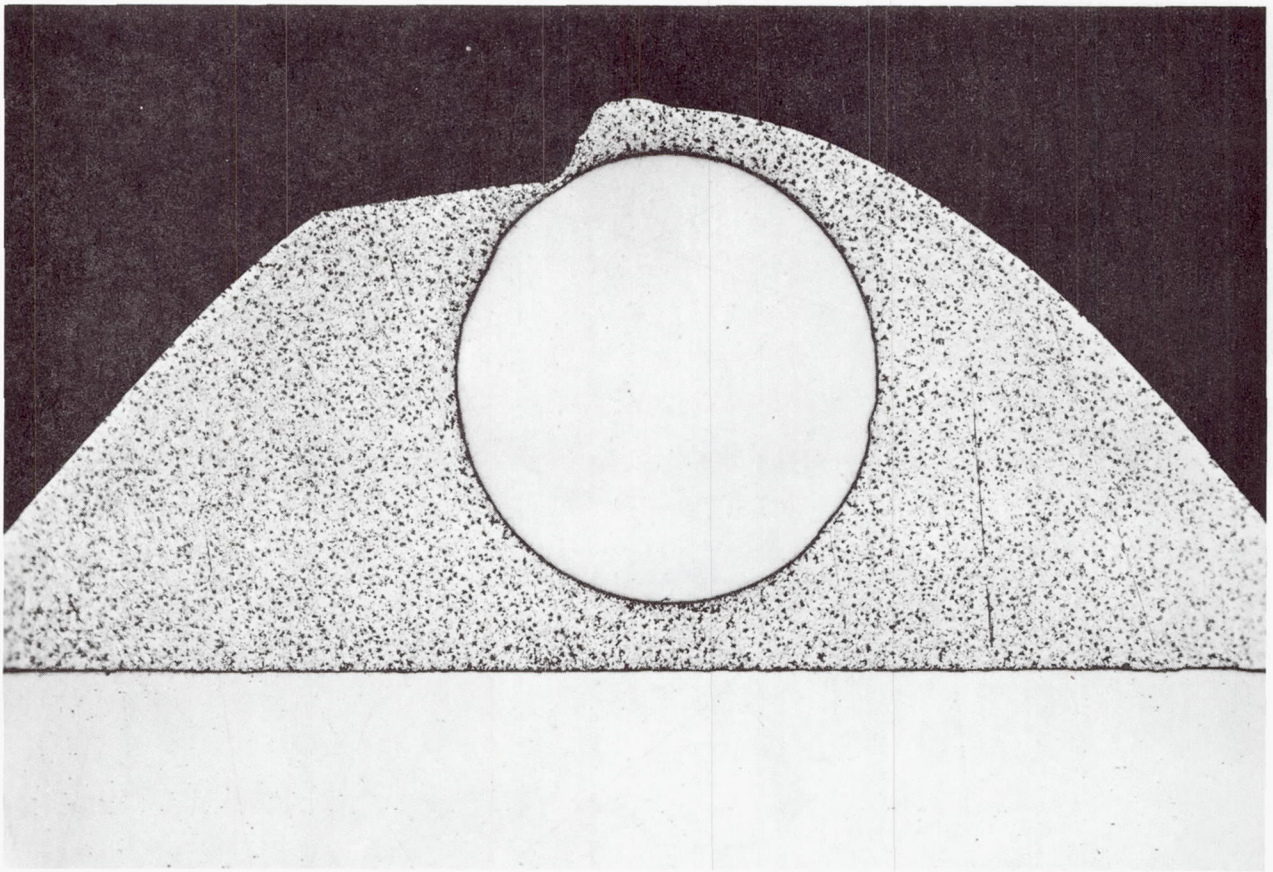


Fig. 7-7 Photomicrographic: Sample No. 10, ETO

Section 8

DIP SOLDERING PROCESS TEST

8.1 APPROACH

Dip soldering process test specimens consisted of a resistor attached to a printed-circuit card segment (Fig. 8-1). As in the case of the hand-soldered items discussed in Section 6, the printed-circuit card specimens and the resistors were grouped as bench-assembled and glove-box-assembled items. The glove-box-assembled items were then divided into sterility-test and performance-test items. All test items were first heat sterilized at 135° C for 24 hr. The resistors were then measured accurately and the assemblies sent to the biological laboratories for inoculation. Following inoculation, the glove-box-assembled specimens and some spare specimens were put in the main glove box (MGB). After sterilization and dip soldering, the sterility test items were transferred to the small glove box (SGB) where they were broken up and placed in jars of culture media. Care was taken to separate the resistor leads from the card. These jars were then taken out of the box and sent to the

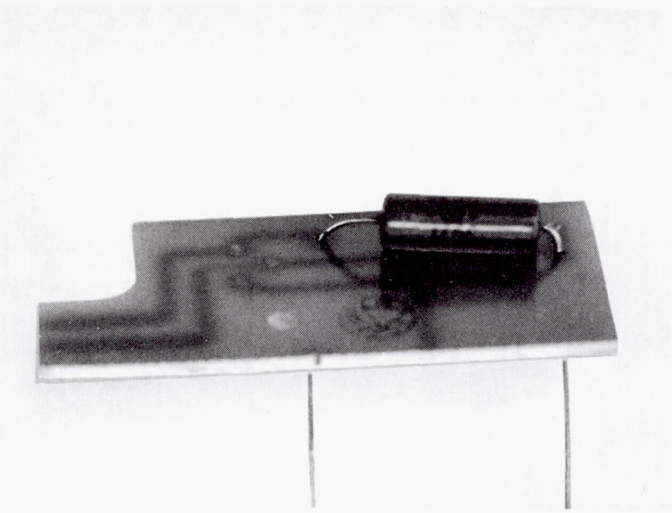


Fig. 8-1 Dip Solder Process Test Specimen

biology labs for incubation and analysis. Following dipping, the performance items were removed from the box and were tested for resistance to determine if the value had been affected.

8.2 PROBLEMS ENCOUNTERED AND SOLUTIONS

Dip soldering in the glove box presented no unusual problems. The actual operation was conducted with about the same ease as was dip soldering on the bench. However, the liquid flux, which was applied to the printed-circuit card with a small paint brush, did not appear to clean the copper as thoroughly as it did on the bench. It was often necessary to make several applications of flux to achieve the required cleanliness. In two instances it was necessary to dip the card specimen twice in order to achieve a complete solder coat.

8.3 OPERATIONAL COMMENTS

It was not necessary to manufacture a special fixture to hold the small card segments. In dipping the cards, the resistor leads were first cut to the proper length and attached to the card. The resistor was then grasped with a pair of needle nosed pliers, and the card was dipped. The card was held in the solder for approximately 3 sec.

It was noted that less scum tended to form on the surface of the solder within the box than formed on the surface of the solder heated on the bench. A block of teflon was used to skim the small amount of scum from the surface prior to dipping.

When measured following dipping, those cards with a high-value resistor (220 kohm) were found to read much lower (about 150 kohm). It was found that flux, some of which remained on the card, was conducting. After the flux was removed, the items were again checked and found to read properly. All boards were washed with alcohol or a detergent solution following assembly operations in all remaining phases of the program.

8.4 RESULTS

The results of the resistance changes of the resistors that had been dipped with the printed-circuit card segment are shown in Table 8-1. These results paralleled those obtained in the hand-soldered process tests. All changes noted are considered to be minor.

Table 8-1

DIP-SOLDERED-RESISTOR VALUES

Resistor	Value Before Assembly (ohm)	Value After Assembly (ohm)	Change (ohm)	Change (%)
P6	26.999	26.989	-0.010	0.037
P8	27.315	27.317	-0.002	0.007
P21	38.870	38.867	-0.003	0.008
P24	39.100	39.088	-0.012	0.031
P42	22.110k	22.100k	-0.010k	0.045
P7	27.127	27.126	-0.001	0.004
P34	39.312	39.315	+0.003	0.008
P61	220.040k	219.980k	-0.060k	0.027
P75	218.570k	218.680k	+0.110k	0.050
P63	219.700k	219.480k	-0.220k	0.100

Three of the sterilization control items were found to be contaminated when biologically tested (Appendix A, page A-5). However, all of the items that were handled in the process test were sterile. As in the case of the contaminated resistor specimen discussed in the previous section, the card specimens were not turned during their soak period. These items were put in small plastic cups and were allowed to stand in the glove box in these cups. It is possible that the gas did not reach the surfaces that touched the plastic and, therefore, these surfaces remained contaminated. All these items were put in the SGB in the cups in which they were sterilized. It is probable that both the

cups and boards were contaminated, and that viable spores were placed in the culture media in the SGB. All specimens prepared for subsequent tests were removed from their containers and hung on nylon cords or were turned at least every 12 hr.

Section 9
NUT-AND-BOLT CONNECTION PROCESS TEST

9.1 APPROACH

A large nut-and-bolt combination consisting of a nut, a bolt, two plates, and a lock washer was selected as the item to demonstrate the ability to make a sterile connection within the glove box. A few trial assemblies were made on the bench that indicated that no undue difficulty would be experienced in working on these items within the glove box. Specimens were divided into glove-box sterility test items and glove-box performance test items. These were heat sterilized, inoculated, and then placed in the main glove box (MGB). All nut-and-bolt test items were sterilized in the disassembled condition. Following the sterilization time period, which in all cases exceeded 48 hr, all specimens were assembled in the glove box. The sterility test specimens were then passed into the small glove box where they were disassembled and placed in jars of nutrient. The glove-box process test items were disassembled within the MGB.

9.2 PROBLEMS ENCOUNTERED AND SOLUTIONS

No performance problems were encountered, as anticipated, in working with this nut-and-bolt combination in the MGB. The test item was not the same nut-and-bolt item used to attach the coil to the printed-circuit card. Smaller nuts and bolts were used for this function. These were assembled in the MGB with the same ease as the larger performance test items.

9.3 OPERATIONAL COMMENTS

It took slightly more time to assemble the nut-and-bolt combination within the glove box system; however, after a few minutes of practice the assembly process was conducted with ease.

9.4 RESULTS

Culturing the items that had been sterilized in the MGB produced no growth. In most of the jars, however, heavy sediment formed that was probably a metallic corrosion product. Microscopic examination and agar plating of each jar showed no growth (Appendix A, page A-5).

At this stage in the program, inoculation of the jars following the incubation period was accomplished with 10^6 spores. At this level of inoculation, growth did not occur and all specimens were considered to be toxic. These nuts and bolts were not used in subsequent card assemblies. The fastener that was used was much smaller and caused no toxicity problems.

Section 10
EPOXY BONDING PROCESS TESTS

10.1 APPROACH

Assembly of spacecraft systems and components sometimes requires the use of epoxy. A test was conducted to explore the effects of sterilized epoxy on bonded specimens.

The test consisted of bonding a metal plate to an identical plate and bonding a metal plate to a segment of a printed-circuit card (see Fig. 10-1). The plate and printed-circuit card segments were first heat-sterilized and inoculated with B. globigii spores.

As the ethylene oxide will not internally sterilize epoxy, a survey was first conducted to locate an epoxy compound in which the base and activator materials could be individually heat-sterilized prior to mixing. Table 10-1 lists the epoxy formulas evaluated. The procedure for evaluation consisted of preparing aluminum weighing cups containing proper amounts of either activator or base materials, sealing each cup with aluminum foil, and heating the cups at 135°C for 24 hours. After the temperature cycle was completed, each epoxy base and activator was examined to determine the heat effects. As shown in the table, only the Rayclad S-1005 epoxy was usable after the heat cycle.

Table 10-1
EPOXY FORMULATIONS EVALUATED

Brand	Type	Effect of Heat
Rayclad	S-1005	No effect
Hysol	K-9-4239	Base hardened, accelerator evaporated
Hysol	Epoxi-Patch	Both accelerator and base hardened
W. T. Bean	R. T. C.	Both accelerator and base hardened

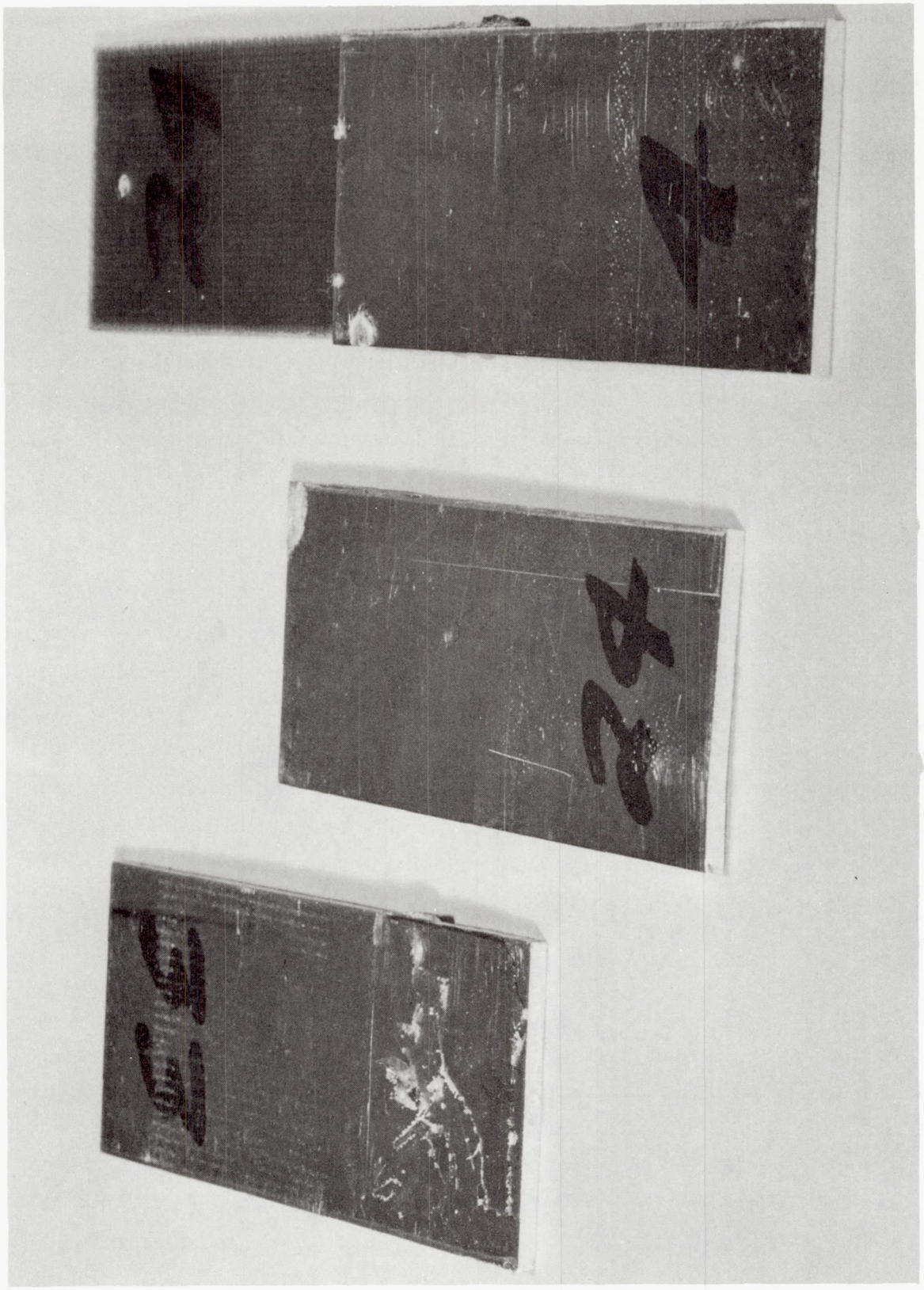


Fig. 10-1 Epoxy Bonding Process Test Specimens

The next phase of the test consisted of heat sterilization of the Rayclad epoxy material. Following heat sterilization, ten of the metal-plate-to-metal-plate asemblies, and ten of the metal-plate-to-printed-circuit-card assemblies were placed in the glove box along with the epoxy containers. After a 48-hour soak in the ETO atmosphere, the epoxy was mixed and the coupons were bonded. A 48-hour ambient cure was allowed for the epoxy. Five coupons of each group of test items were sent to the LMSC test laboratory for pull tests on a Tinius-Olsen tensile test machine. The remaining coupons were subjected to sterility testing. These coupons were broken apart and the epoxy scraped into the culture jars. The remaining epoxy was removed from the glove box and used to bond additional bench performance test assemblies. In addition, fresh epoxy was prepared and used to bond performance test coupons.

As a further test of bonding strength, ten pull-test specimens were prepared using heat-sterilized epoxy which was not subjected to ETO.

10.2 PROBLEMS ENCOUNTERED AND SOLUTIONS

The application of epoxy could be a messy operation. The mixed epoxy could not be prevented from contacting the gloves. Disposable outer gloves should be worn when epoxy bonding is routinely accomplished in a glove box. The epoxy which was deposited on the gloves during the operation was easily removed after it had dried. However, care had to be taken to prevent the finger of the gloves from being cemented together as the epoxy cured.

10.3 OPERATIONAL COMMENTS

In general, operations took three times as long in the glove box as when performed on the bench. This was caused mainly by the loss of sense of touch when using the gloves It also affected the workmanship of the bond. Both of these conditions improved as the operator became more proficient with glove box techniques.

10.4 RESULTS

Table 10-2 summarizes the results of the epoxy bonding process test. It presents in matrix form the "break away" force values obtained using the tensile test machine. Column headings indicate the treatment received by the Rayclad epoxy and the locations (main glove box or bench) at which the epoxy formula was applied to the test coupons. The two types of materials which were bonded together using the epoxy are indicated by row headings; (1) aluminum metal plate to aluminum metal plate, or (2) aluminum metal plate to printed-circuit card stock (fiberglass). The mean value of the force readings taken and the range of the readings is also shown in each block of the matrix.

The mean values show an interesting relationship. The highest breakaway force was obtained using heat-sterilized epoxy, and was over 300 lb greater than the nonsterilized epoxy in the case of the plate-to-plate specimens, and over 350 lb greater in the case of the plate-to-printed-circuit card specimens. The heat-and gas-sterilized test items assembled in the main glove box have mean breakaway force values greater than the nonsterilized epoxy in both cases. These nonsterilized epoxy values are lower, but within 90 lb of the mean values of the bench-assembled items that were bonded using the same heat- and gas-sterilized epoxy.

The ranges of the breakaway force readings between the test locations is also significant. The greatest ranges in values occurred in both cases among the group of test specimens bonded in the main glove box. The dispersion in readings between samples as indicated by the wide range of these values is probably accounted for by an inexperienced assembler using gloves. It is to be expected that a reduced range of values and, hence, a more uniform product would be achieved as additional specimens are bonded.

It can be concluded from the above data that equal bond strengths can be achieved using heat- and gas-sterilized epoxy as can be obtained using the same nonsterilized formulation.

Table 10-2

EPOXY BONDING PROCESS TEST (BREAKAWAY FORCE IN POUNDS)

Type of Bonding	Nonsterilized Epoxy (Bench) Values	Heat-Sterilized Epoxy (Bench) Values	Heat- and Gas-Sterilized (Main Glove Box) Values	Heat- and Gas-Sterilized (Bench) Values
Plate-to-Plate	576 538 580 618 838 Mean = 630 Range = 300	980 836 806 1,270 895 Mean = 957 Range = 464	818 978 554 796 618 1,000 726 452 502 398 770 Mean = 692 Range = 602	808 840 672 712 820 Mean = 770 Range = 168
Plate-to-Printed-Circuit-Card	302 322 372 350 Mean = 337 Range = 70	654 435 975 786 Mean = 713 Range = 540	700 506 342 428 630 284 436 622 978 Mean = 547 Range = 694	560 720 415 740 730 Mean = 633 Range = 325

The results of the sterilization assays conducted on the epoxy-bonded items were disappointing. Of five metal-plate-to-metal-plate test items, two were contaminated and three did not produce growth when the media was inoculated following the seven-day incubation period. Of seven jars containing metal-plate-to-printed-circuit card test items, all were toxic. (Detailed results are contained in Appendix A, pages A-6 and A-7.) These results, and similar results obtained in the staking test, initiated the first investigation of sterilization skip causes discussed in Section 15.

Section 11
POTTING PROCESS TESTS

11.1 APPROACH

The procedure used for the potting-process test was similar to that employed in the epoxy tests described in Section 9. In the first phase of the test, Dow Corning WS 1359 compound base and accelerator were heated at 135°C for 24 hours to determine the effects of this cycle on the constituents. Both base and accelerator took the heat without apparent degradation.

Resistors were first mounted on a printed-circuit card segment and covered with the compound (see Fig. 11-1). At Jet Propulsion Laboratory's suggestion, additional resistors were potted in a cylindrical rubber sleeve. In both cases, the potting compound ingredients used in the main glove box (MGB) were individually heat sterilized in covered aluminum cups. After the heat sterilization, the compound was gas sterilized for a 48-hour period. The compound was then mixed and the potting operation

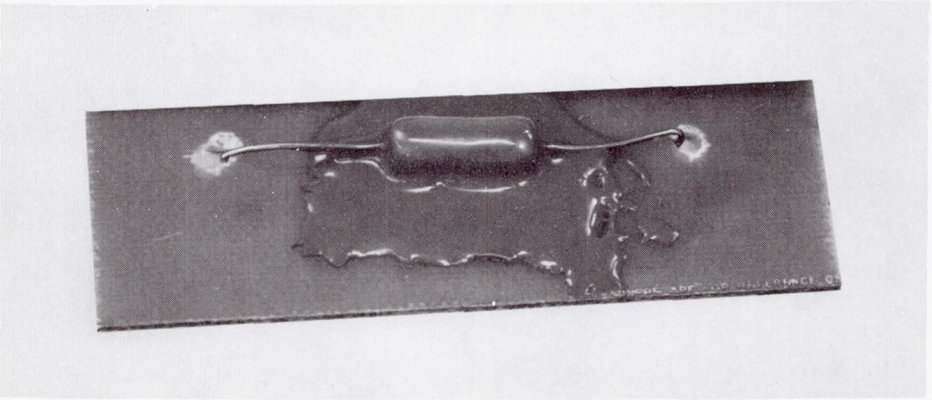


Fig. 11-1 Sterilized Potting Compound on Resistor

performed. Remaining compound was removed from the glove box and used to pot bench test items in the laboratory atmosphere. As a further test, fresh compound was mixed and used to pot additional units on the bench. No attempt was made to inoculate the potting compound.

11.2 PROBLEMS ENCOUNTERED AND SOLUTIONS

Previous tests by LMSC biologists have shown that it is difficult, if not impossible, to recover spores from rubber-like substances. It was, therefore, determined that conclusive sterility checking could not be achieved. Concurrence with this conclusion was obtained from Jet Propulsion Laboratory personnel.

11.3 OPERATIONAL COMMENTS

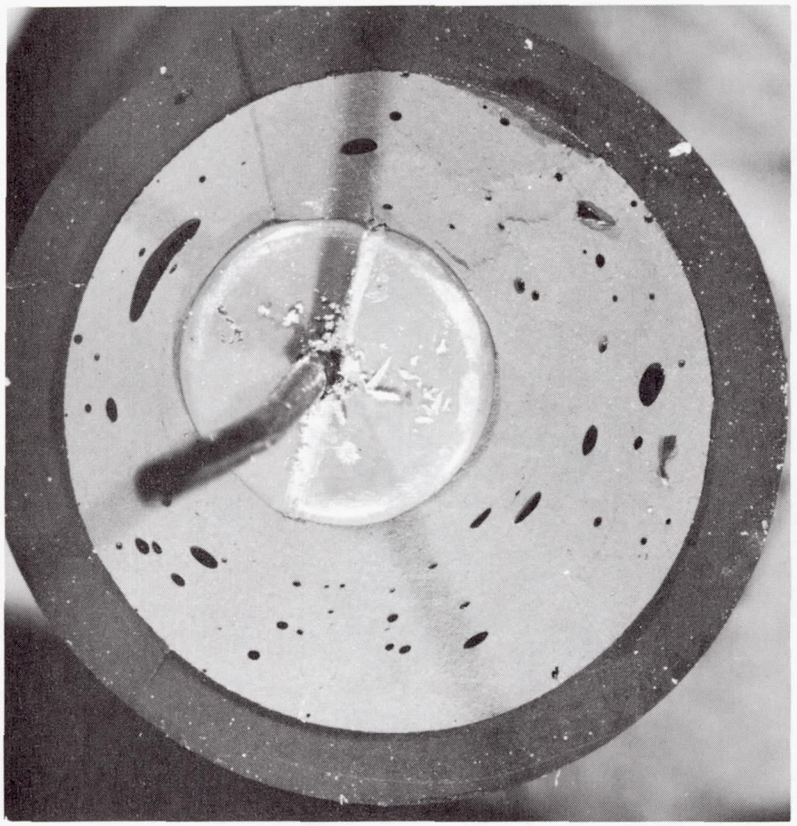
As discussed in the section on epoxy bonding, working with substances which require mixing and application is a messy task. As before, it is recommended that disposable gloves be utilized when working with substances of this sort.

11.4 RESULTS

No performance tests of the initial items potted at the various locations were attempted. Visual examination of the compound, however, revealed no apparent difference between sterilized and nonsterilized compound.

Photomicrographic analyses of the resistors potted in rubber sleeves was accomplished after the sleeves were sectioned. Five resistors were potted using WS 1359 compound which had not been sterilized, 5 with compound which was heat-sterilized, and with a compound which was both heat and gas sterilized. Figures 11-2 through 11-4 are photomicrographs of typical resistors from each group.

Small porosity was evident in all three groups, and large voids were found in the unsterilized and heat-sterilized (only) specimens. The heat-and-gas-sterilized specimens appeared more dense and uniform than either the unsterilized or heat-sterilized



8×

Fig. 11-2 Potting Process Test Specimen—Compound Not Sterilized

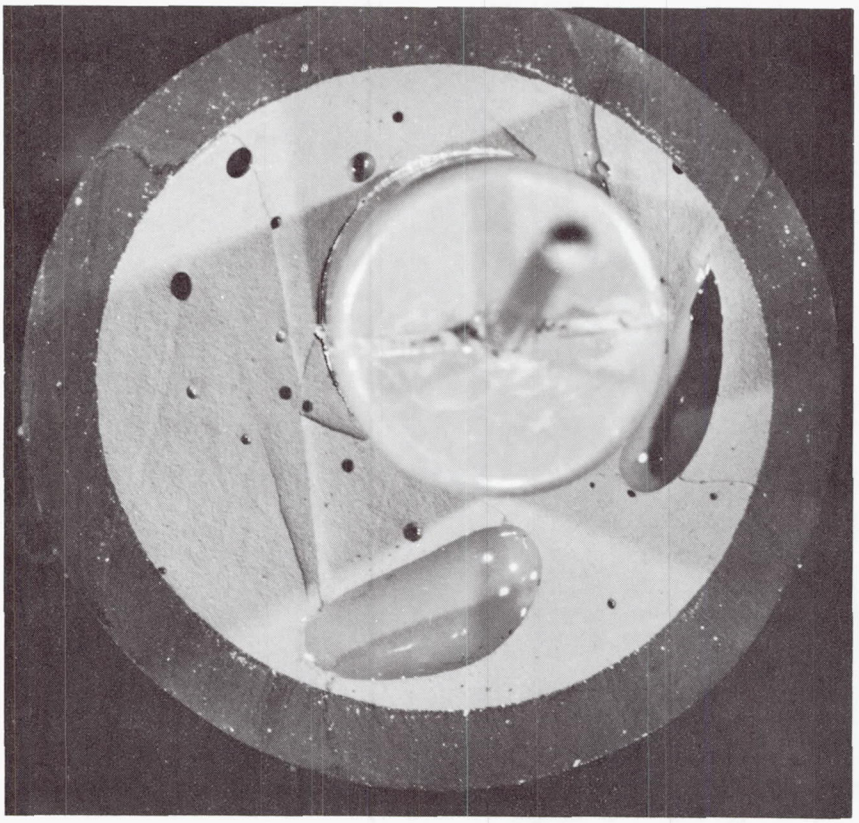


Fig. 11-3 Potting Process Test Specimen—Heat Sterilized

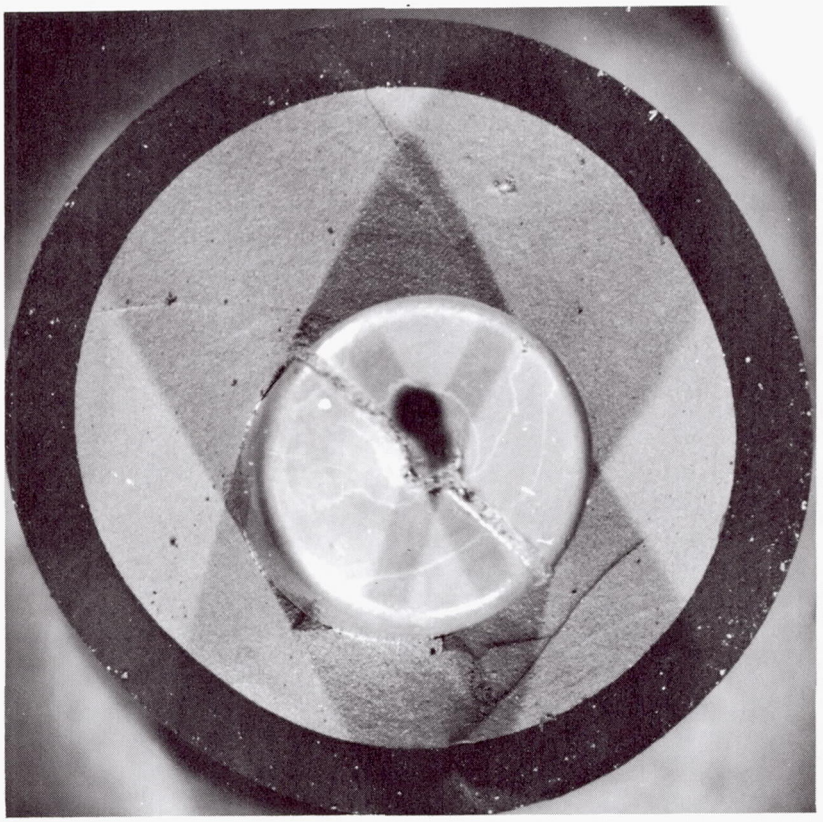


Fig. 11-4 Potting Process Test Specimen – Heat and Gas Sterilized

items. The reason for this is probably related to the learning curve rather than to any property of ETO which enchances the potting process,because the MGB assembled heat-and-gas-sterilized items were the last of the series potted. The tests do show that it is feasible to pot items with compounds which have been heat and gas sterilized.

The initial test specimens were cultured. One of three proved to be nonsterile. Detailed information is presented in Appendix A. page A-6.

Section 12
STAKING PROCESS TEST

12.1 APPROACH

In this phase of the program a terminal was attached to a printed-circuit-card segment using a swaging machine. This machine requires a simple motion of one hand. The card segment and terminal are shown in Fig. 12-1. The hole in the printed-circuit card in which the terminal is mounted was drilled prior to heat sterilization and inoculation.

12.2 PROBLEMS ENCOUNTERED AND SOLUTIONS

Work done on the bench using the swaging tool indicated that no difficulty would be experienced when working in the main glove box. As anticipated, no problems occurred. It took approximately 8 minutes to attach 15 terminals.

12.3 OPERATIONAL COMMENTS

All 15 items were transferred to the small glove box where the terminal was carefully removed from the printed-circuit card. This was accomplished using tin shears. A cut was made in the card to the point of terminal attachment. Needle nosed pliers were then used to pull the terminal away from the hole in the card. The card, terminal, and any residue from the shearing operation were placed in jars of nutrient and removed from the glove box system.

12.4 RESULTS

As stated in 12.2 above, no problems were experienced in performing the staking operation in the main glove box. Eight of the printed circuit cards and terminals were placed

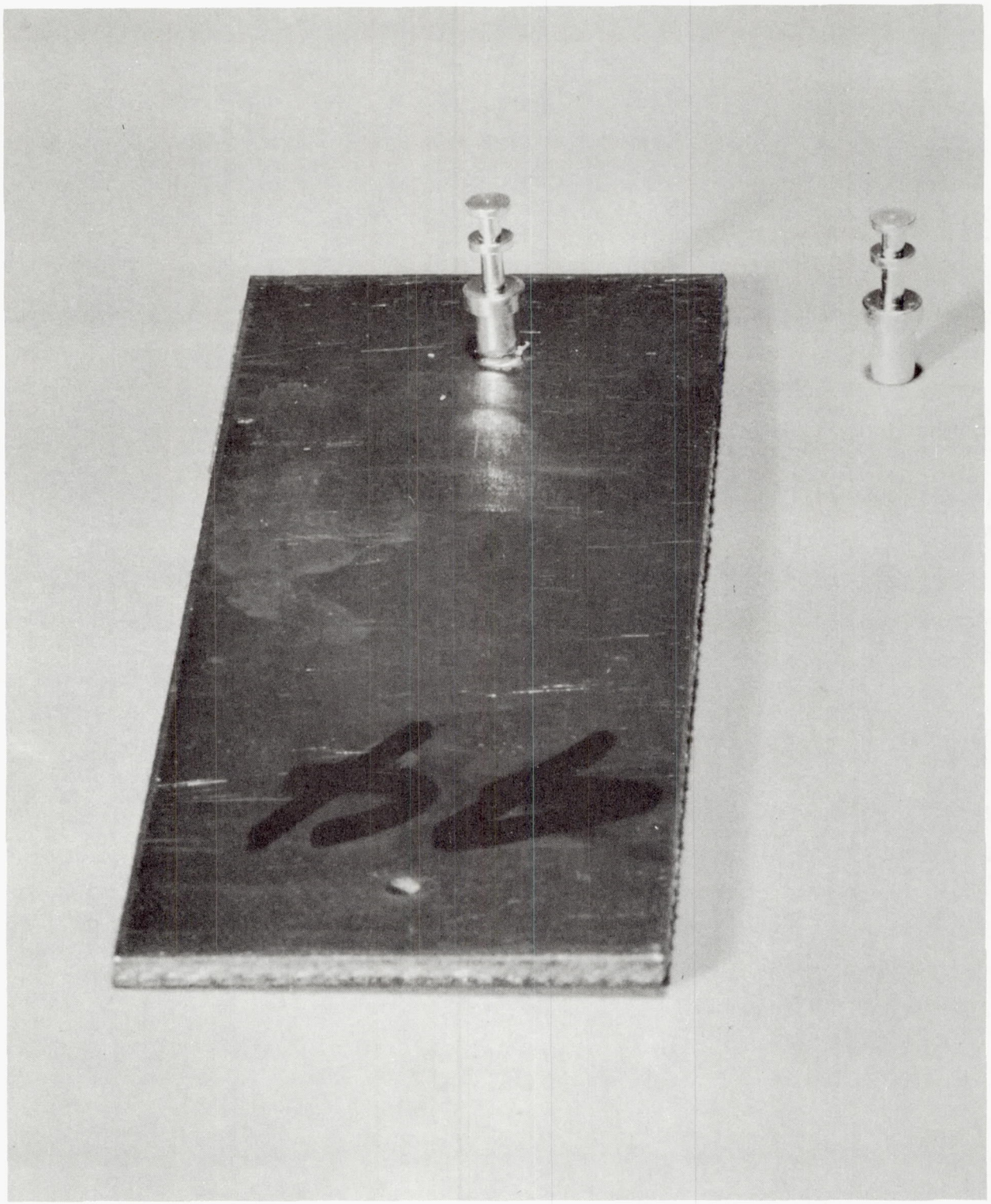


Fig. 12-1 Staking Process Test Specimen

in one jar of nutrient and seven in another jar. The jar containing the eight test items proved to be toxic, and no determination as to the sterility of its contents could be made (Appendix A, page A-6). The toxicity problem is discussed in Section 5 of this report. In this instance the toxicity of the nutrient was probably caused by the uncoated copper on the printed circuit card segment. The second jar was contaminated. This sterilization skip and those occurring in the epoxy bonding test initiated the first contamination-cause investigations discussed in Section 15.

Section 13
BREADBOARD ASSEMBLY TEST

13.1 APPROACH

In the breadboard assembly test phase of the study, a complete test circuit was first assembled on the bench. The circuit components were hand soldered to terminal posts (Fig. 13-1) rather than attached by dip soldering as on the final printed circuit card. As the components are less densely packaged in this breadboard configuration, circuit assembly is easily accomplished. Following assembly on the bench, readings of circuit values were taken using the automatic checkout equipment (ACRE). Circuits were then disassembled by unsoldering the component's leads from the terminal posts. All components and the breadboard jig were then placed in the main glove box (MGB) where they were allowed to soak in ETO for at least 48 hours. Circuits were then re-assembled on their breadboard jigs in the MGB, in ETO. Each component was hand soldered to the same terminal post to which it had been attached when previously assembled on the bench. Circuits were then again automatically tested using ACRE. Following this check, the circuits were removed from the MGB and tested on the bench.

13.2 PROBLEMS ENCOUNTERED AND SOLUTIONS

No problems were encountered during assembly of breadboards either on the bench in the laboratory atmosphere, or in the main glove box in ETO.

13.3 OPERATIONAL COMMENTS

Several readings using ACRE were made at each assembly point. This was done in order that truly representative values would be obtained at each stage of manufacture.

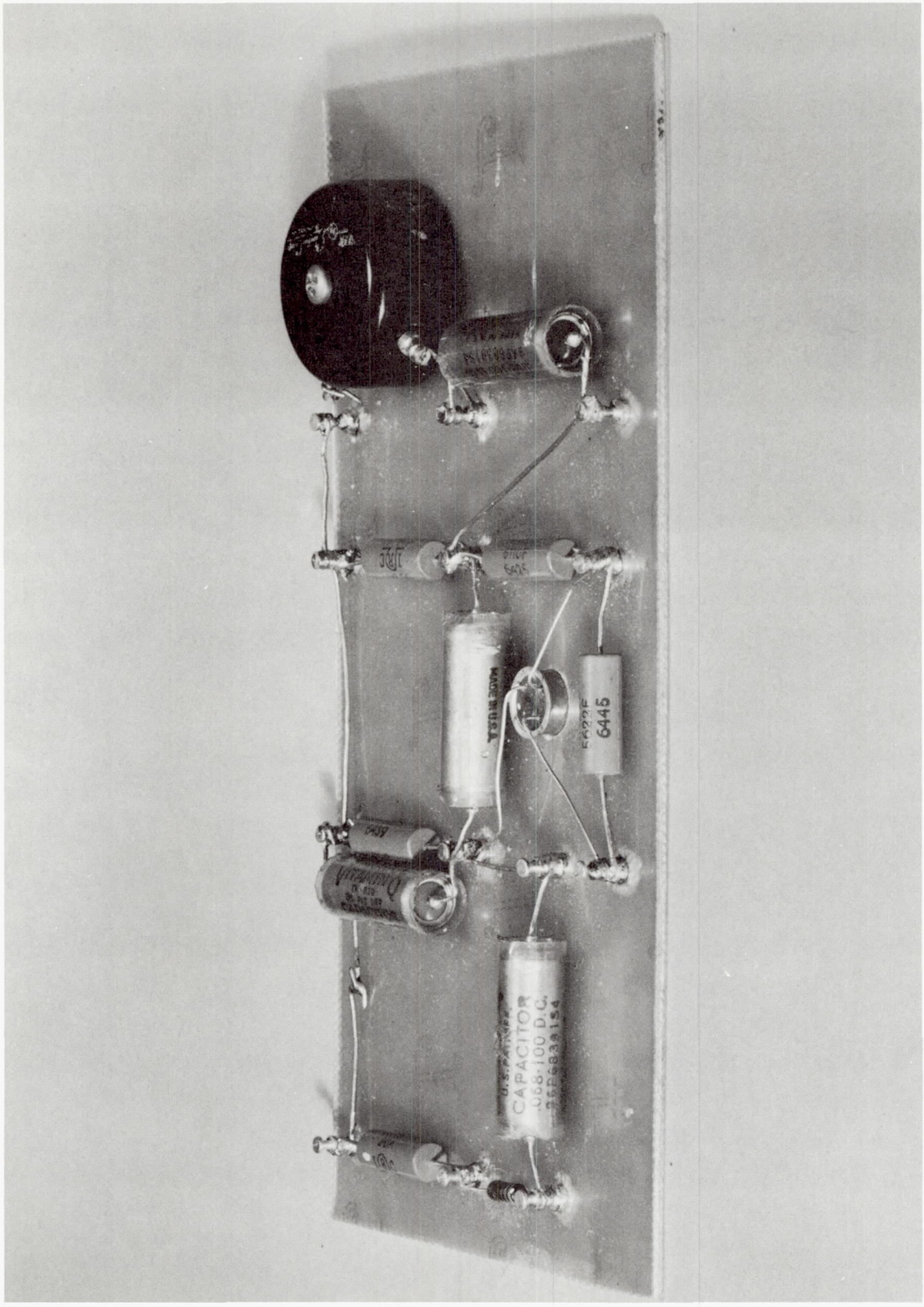


Fig. 13-1 Circuit Breadboard

13.4 RESULTS

Tables 13-1 and 13-2 show the readings obtained from Breadboard Circuits No. 1 and No. 2 respectively. In both cases the circuits show slight changes between the bench and glove box readings. However, the changes on Breadboard Circuit No. 2 are considerably less than on Breadboard Circuit No. 1. The second circuit was manufactured late in the program at a time when the operator's proficiency was at its peak.

The slight changes noted in readings between locations on the two breadboards are not considered to reflect degradation because of manufacture in the ETO atmosphere.

Page intentionally left blank

Table 13-2

BREADBOARD CIRCUIT NO. 2 VALUES READ

Date	Location	Run No.	Test Point No.									
			7	8	9	10	11	12	13	14	15	16
2-25	Bread-board bench	1	961	833	824	581	657	605	545	550	887	741
		2	961	834	825	590	656	605	545	550	886	741
		3	961	834	824	580	656	605	545	549	886	741
		4	961	833	824	581	656	605	545	550	886	742
		5	961	834	825	581	655	604	544	550	888	741
		Mean	961	834	824	582	656	605	545	550	887	741
3-1	Bench	1	962	835	825	581	655	606	547	552	890	741
		2	962	834	825	581	651	608	546	552	890	742
		3	962	834	826	581	651	606	548	551	890	742
		4	962	835	826	581	652	606	566	551	889	741
		5	962	835	825	580	651	606	546	551	889	742
		Mean	962	835	825	581	652	606	550	551	890	742
3-5	MGB after ETO soak	1	962	834	825	581	672	605	546	550	889	740
		2	962	834	825	582	672	606	546	551	891	740
		3	963	835	826	581	673	607	546	552	891	739
		4	962	834	826	581	673	607	547	551	891	739
		5	963	835	826	582	673	607	547	552	891	740
		Mean	962	834	826	581	673	606	546	551	891	740
3-8	Bench	1	962	834	825	584	665	608	548	553	893	741
		2	963	835	826	584	664	608	548	553	894	741
		3	962	835	826	584	664	608	548	553	893	741
		4	962	835	826	584	664	608	548	553	892	741
		5	962	835	826	584	663	608	548	553	893	741
		Mean	962	835	826	584	664	608	548	553	893	741

Section 14
PRINTED-CIRCUIT-CARD ASSEMBLY

14.1 APPROACH

14.1.1 General Sequence

The Experimental Study of Sterile Assembly Techniques approached the determination of sterile assembly feasibility by the actual assembly of electronic circuits in a glove box system containing atmospheres of ethylene oxide/Freon 12 (ETO), sterile nitrogen, and sterile air. Prior to assembly in these atmospheres, all of the circuit components received a sterilizing treatment in ETO. Figure 14-1 shows the assembled circuit. To test the effect of the sterilizing atmosphere, circuit components were first heat sterilized and then inoculated with a known bacterial spore preparation prior to ETO exposure.

A circuit was selected, for the study, that would be reasonably sensitive to changes in component values caused by exposures to the ETO. The design and selection of components for this circuit are discussed in subsection 14.1.2.

When circuit components were received, they were divided into circuit groups. The individual components were marked to assure that at all stages of assembly they remained associated with their group and retained their same positions in the circuit. The circuit components and printed-circuit cards were then heat sterilized and inoculated.

Following inoculation, all printed-circuit card components were first assembled on the bench in the laboratory environment by clamping the components in their proper circuit relationships to a terminal board (Fig. 14-2). Data were then taken on all cards using the automatic checkout equipment (ACRE). At least three ACRE runs were made to eliminate any spurious values caused by noise spikes, and a representative run was selected for use in subsequent evaluations. This procedure was followed

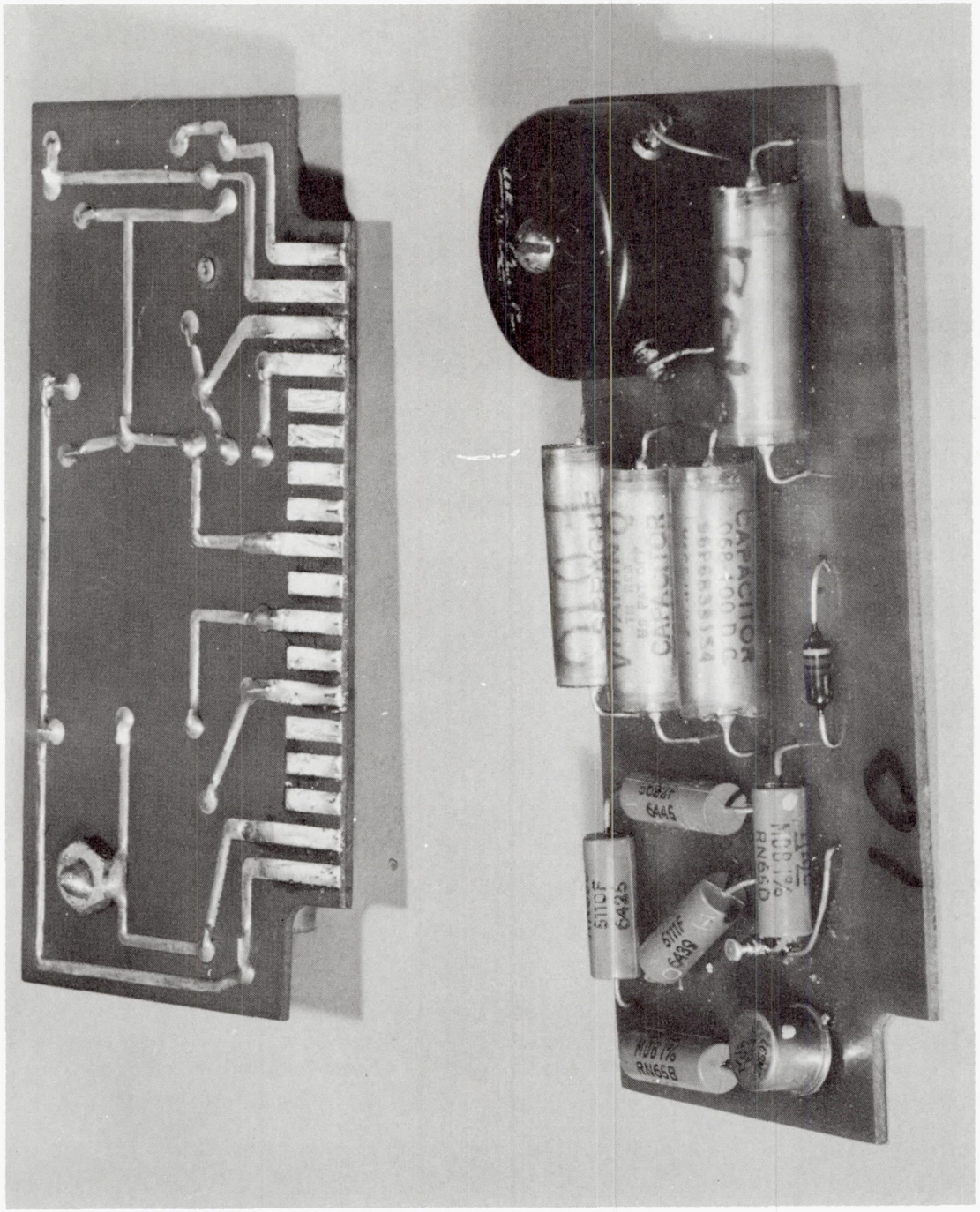


Fig. 14-1 Study Test Circuit

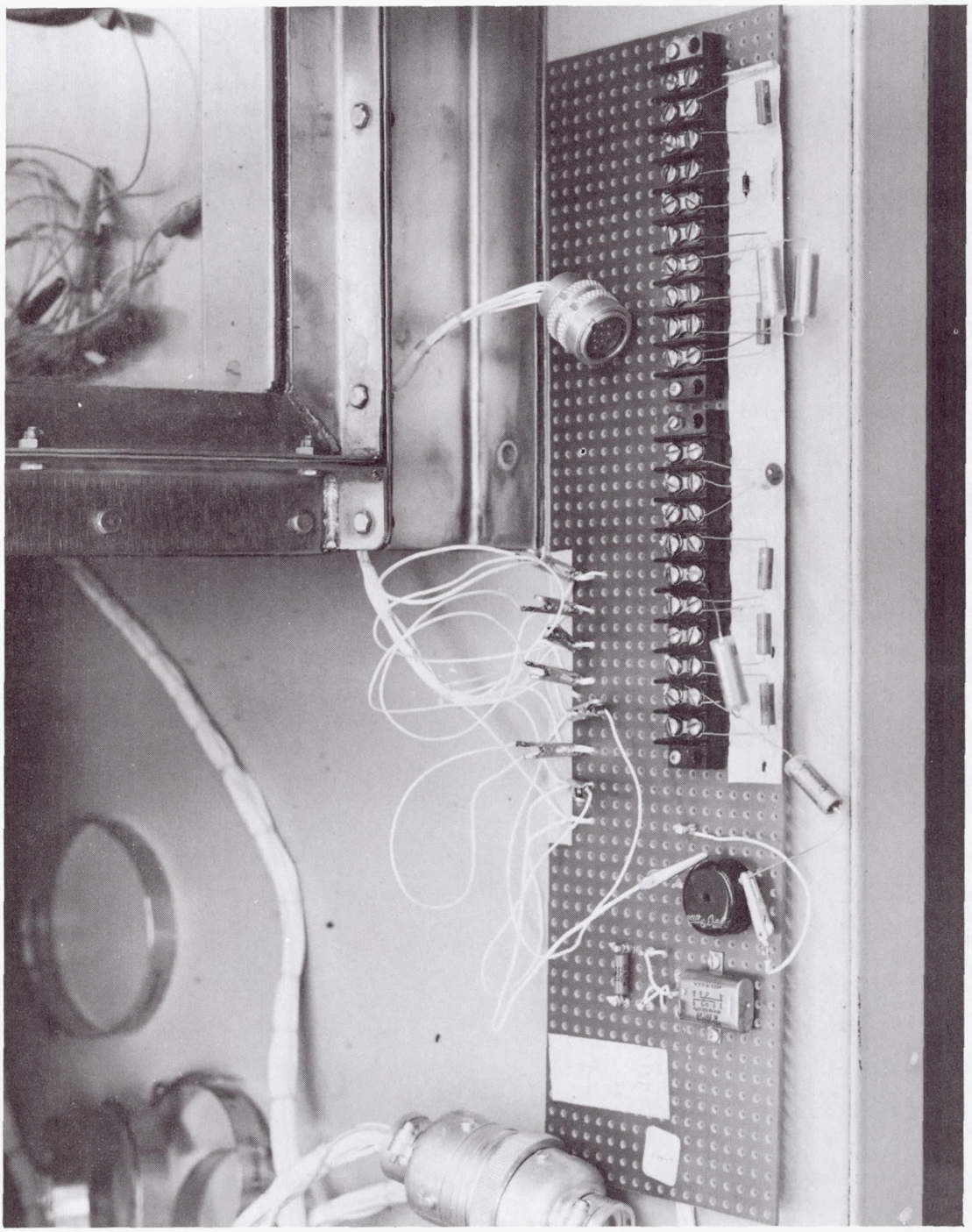


Fig. 14-2 Preassembly Test Terminal Board

at all points in the test sequence where data were collected. Selected cards were next assembled on the bench using normal dip soldering, hand soldering, and terminal attachment techniques. Practice runs had previously been made to ensure that the operator was capable of assembling as good a card in the glove box as could be made on the bench. These practice cards were cut up and bottled as sterility test items or were discarded.

The next stage for cards that were to be assembled in ETO, sterile nitrogen, and sterile air was an ETO soak treatment in the main glove box for at least 48 hr. These cards were then assembled (hand soldered, dip soldered, terminal attached, etc.) in the main glove box (MGB) in an ETO atmosphere. After the atmosphere and the card had cooled, excess solder flux was removed from the dipped side of the card. The first post-assembly ACRE measurements were then made on the card. After at least a day of continued exposure to the ETO, another set of data was taken. Corresponding sets of data were collected from the control group of cards that had been assembled on the bench in the laboratory environment.

The cards assembled in sterile nitrogen and sterile air were transferred, prior to assembly and following ETO sterilization, to the small glove box (SGB). This box was then flushed with sterile nitrogen (or sterile air) and the assembly operation was performed. Two sets of ACRE measurements were taken as they had been on the ETO- and bench-assembled cards.

An additional task was to determine card performance when sealed in an atmosphere of the gas in which they had been assembled. Five bench-, five ETO-, five sterile-air-, and five sterile-nitrogen-assembled cards were placed in gasketed cans and sealed in their respective atmospheres (Fig. 14-3). The cans were each provided with a hermetically sealed connector that allowed ACRE to collect data from the encapsulated cards. Two sets of data were collected, one shortly after sealing and another at least a day following the first "canned" run.

Following the last data collection run on the card groups, the cards were cut up and placed in jars of culture media. They were then sent to the LMSC Palo Alto biology

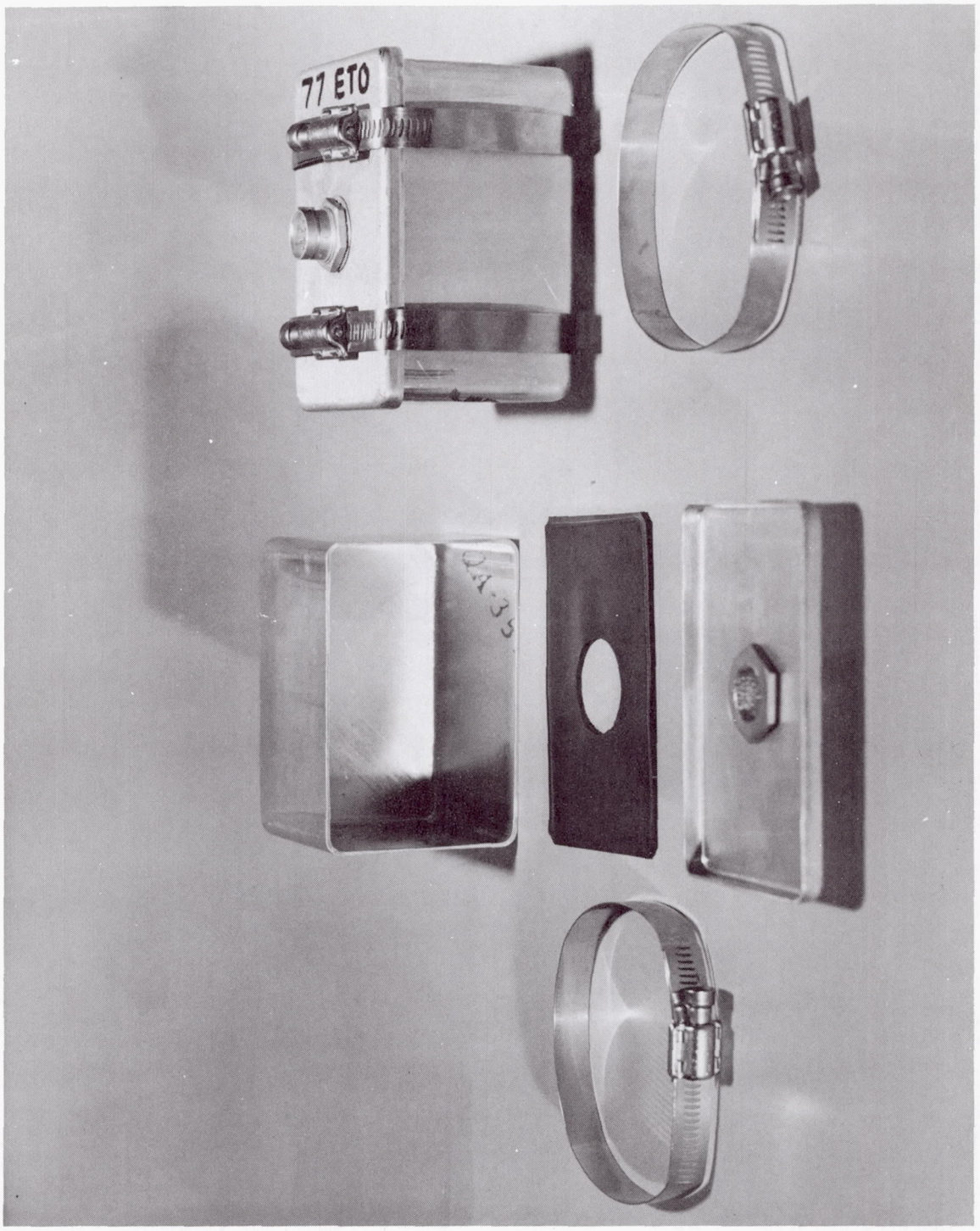


Fig. 14-3 Sealed Can for Printed-Circuit Cards

laboratories where they were placed on a shaker and incubated for seven days. After this time period the contents of the jars were visually examined for turbidity, their contents were evaluated microscopically, and some of the nutrient was placed on agar plates to check for the characteristic orange colonies of B. globigii. The jars that gave no indication of contamination were then inoculated with 10^2 spores and checked for growth to verify that the media were not toxic.

During the printed-circuit-card phase of the program, 78 cards were assembled. The path of assembly and ultimate disposition of each card are shown graphically in Fig. 14-4.

14.1.2 Test Circuit

An oscillator circuit was chosen for assembly and evaluation. A diagram of the circuit is shown in Fig. 14-5. This particular circuit provided the following advantages for the study:

- It was responsive to small changes in component values that might occur because of the effects of the ETO.
- It contained five typical types of electronic components: resistors, capacitors, a diode, a transistor, and a coil.
- It was compatible with the measurement capability of the ACRE automatic checkout equipment that was available for use on the program.
- It could be dip soldered on a small printed-circuit card with a packing density representative of most such circuits.
- Components could be obtained for a reasonable cost that would withstand the dry heat sterilization cycle (135° C for 24 hr).

Table 14-1 describes the components used in the circuit. The printed-circuit card upon which the oscillator components were mounted was designed at Lockheed Missiles & Space Company and was manufactured by CIRCO Company of Palo Alto, California. The card material was one-sided epoxy glass (G-10-1/2), 3/64-in. thick, with 2 oz of copper.

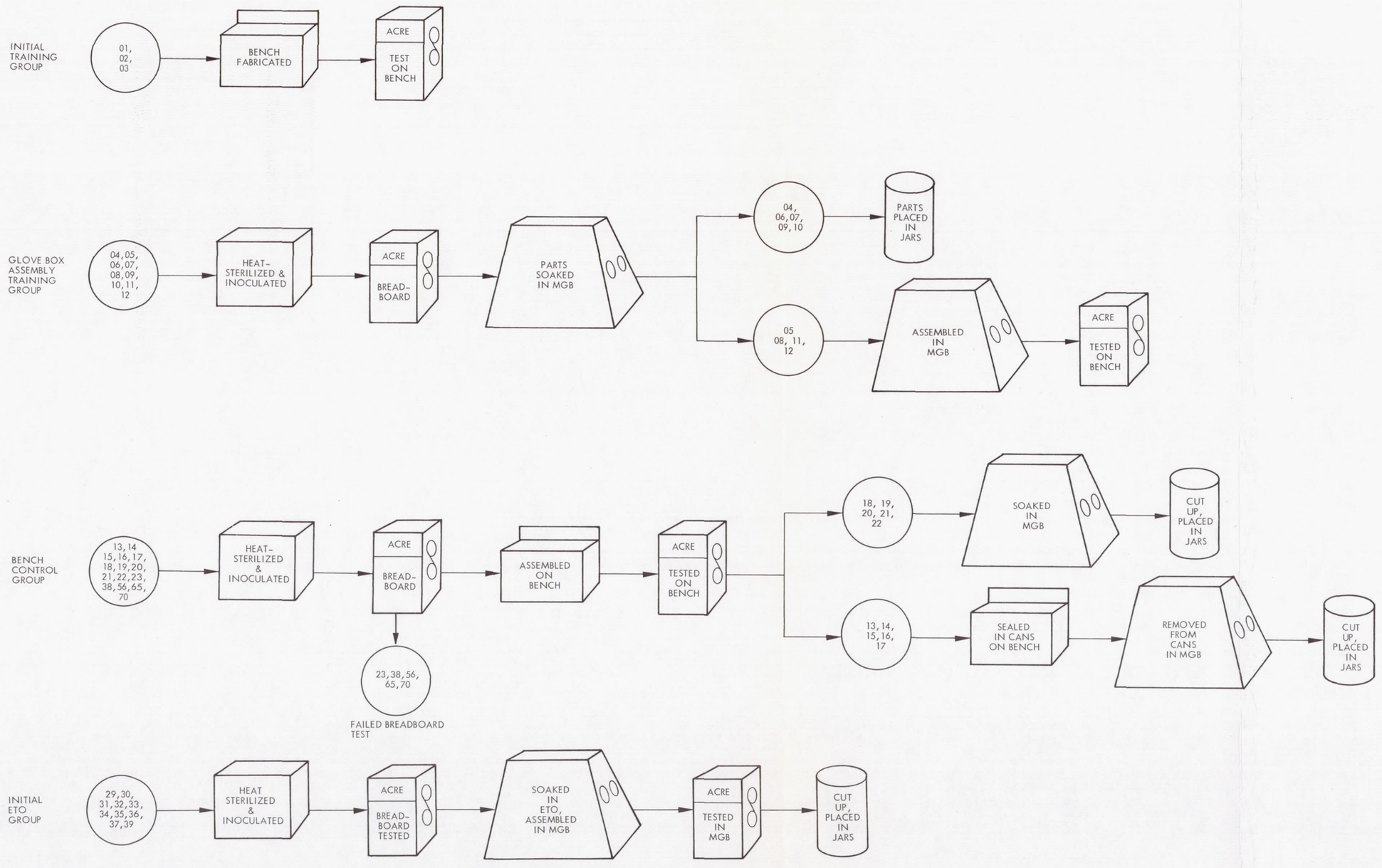


Fig. 14-4 Circuit Assembly Flow Schematic

Page intentionally left blank

1727

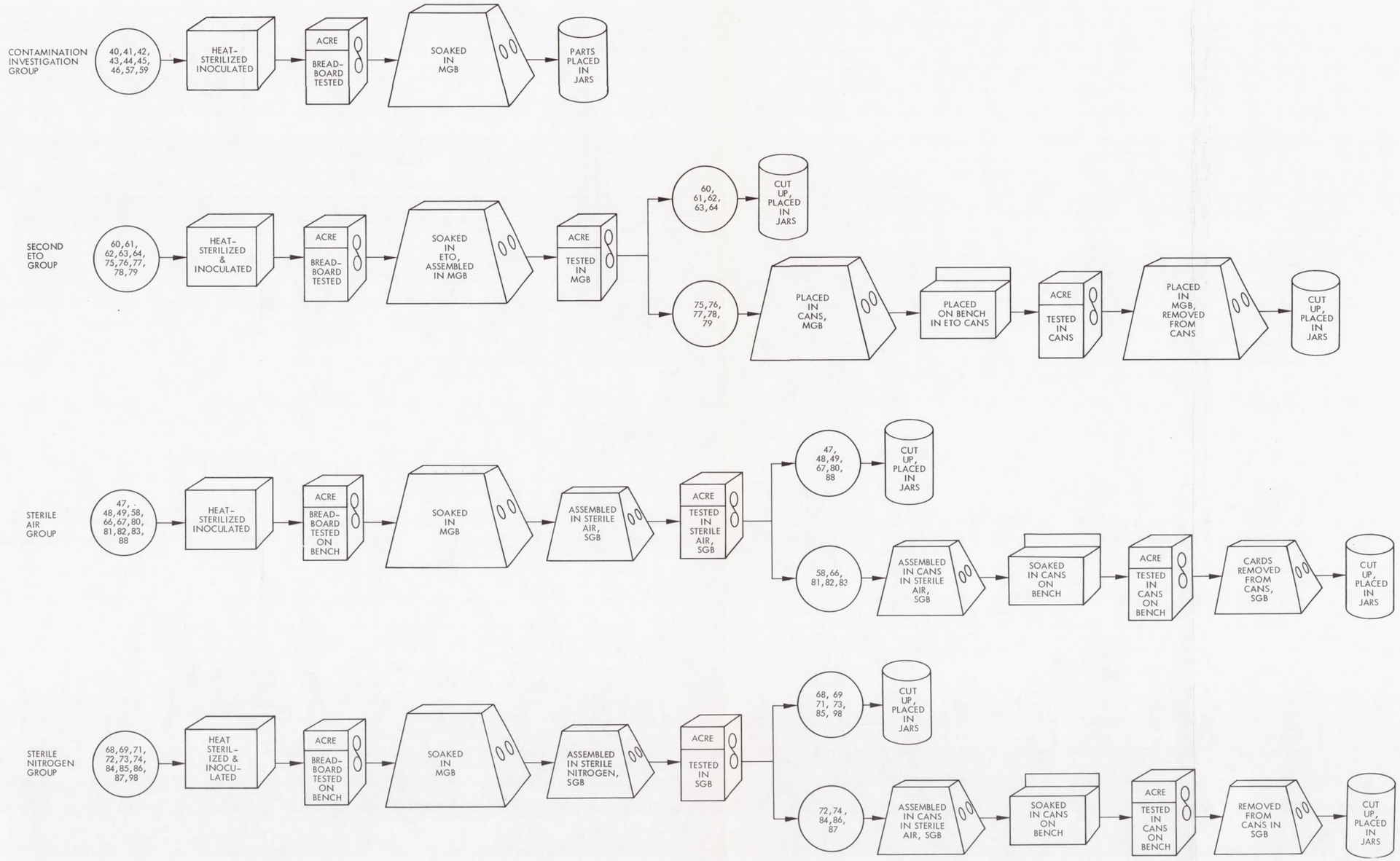


Fig. 14-4 (Continued)

Page intentionally left blank

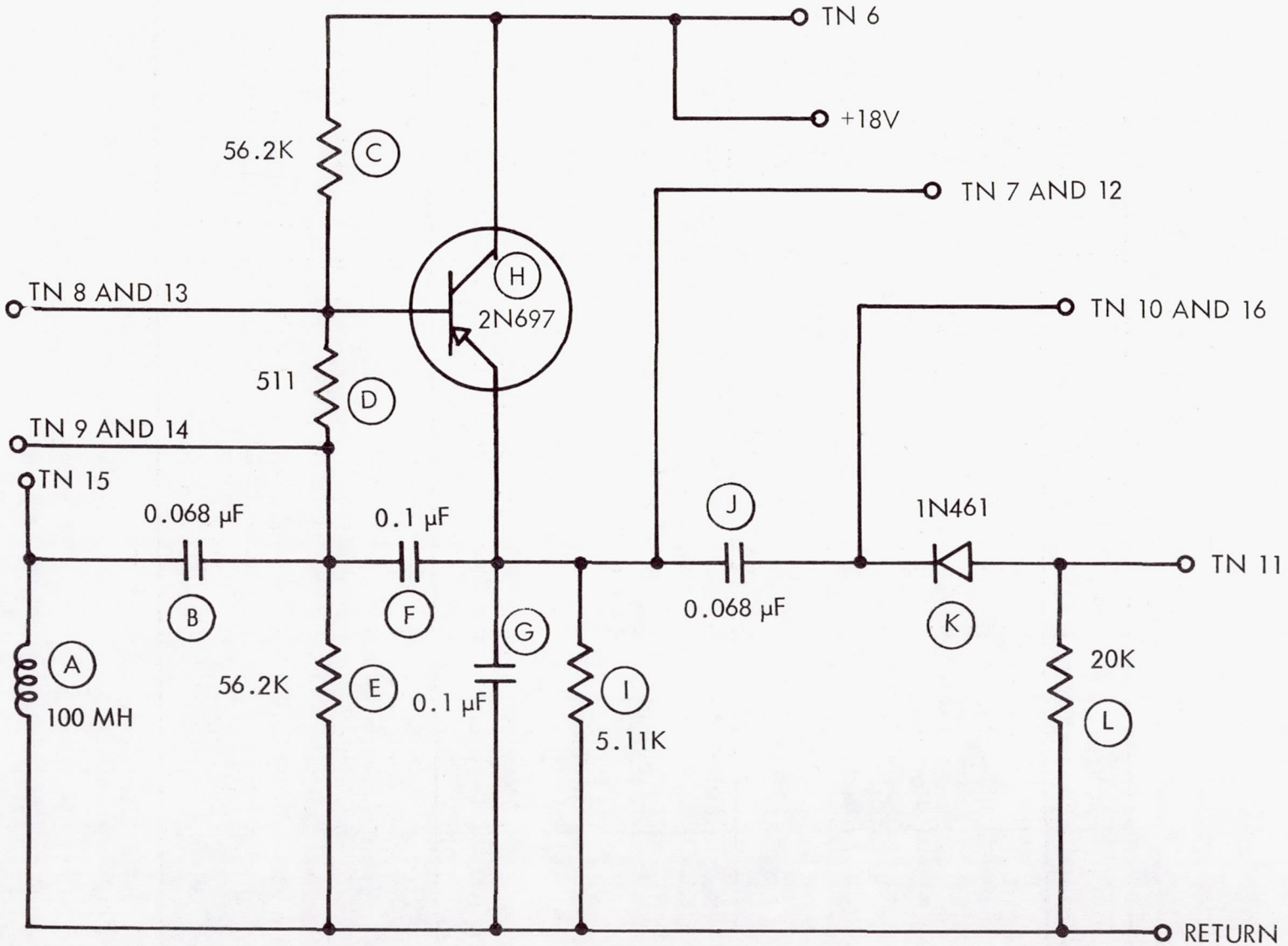


Fig. 14-5 Test Circuit Including ACRE Test Points (TN)

Table 14-1

COMPONENT DESCRIPTIONS

Letter Designation*	Type Component	Description and Specification	Manufacturer
A	Coil TM 100A	100 mh, 32.5 ohm MIL-T-27A	Chicago Stancor
B	Capacitor Vitamin Q Subminiature	0.068 μF, Series 96P MIL-C-25	Sprague
C	Resistor	56.2 kohm, 1/2 w MIL-R-10509C-RN65B	International Resistance Co.
D	Resistor	511 ohm, 1/2 w (same as C)	Same as C
E	Resistor	56.2 kohm, 1/2 w (same as C)	Same as C
F	Capacitor	0.1 μF (same as B)	Same as B
G	Capacitor	0.1 μF (same as B)	Same as B
H	Transistor	2N697, NPN planar passivated beta spread 40 to 120 MIL-s-19500	Texas Instruments
I	Resistor	5.11 kohm (same as C)	Same as C
J	Capacitor	0.068 μF (same as B)	Same as B
K	Diode 1193A	1N461, silicon MIL-s-19500	Texas Instruments
L	Resistor	20 kohm (same as C)	Same as C

*See Fig. 14-5.

The aluminum cans in which the printed-circuit cards were sealed for the last portion of the program were manufactured by Moore-Lee Company. The boxes were 4-3/4 by 3 by 3 in. They were fitted with loose. gasket fitting covers that were tightened with two each Aero-Seal model QS 200 screw-driver-tightened clamps. The hermetically sealed connector in the cap was a Deutsch 12-pin model DM5605.

14.1.3 Automatic Checkout of Test Circuit

The ACRE equipment was used to supply 18 vdc power to the card and to record values from test points across each circuit component. These test points were brought out to the "tuning fork" contacts on the card. Signals from these test points were delivered via a test fixture, consisting of a standard box mounted printed-circuit card connector and cable assembly at each testing location, to the ACRE test station. The first five tests performed by ACRE were self tests of its own operational readiness. the sixth was a test of the input to the card. and the seventh through sixteenth were tests on the card. All measurements were made between a point on the card and ground. A description of each test and the test number are presented in Table 14-2.

14.2 PROBLEMS ENCOUNTERED AND SOLUTIONS

14.2.1 Dip Soldering

Dip soldering a printed-circuit card proved to be a more complex operation than dipping a small card segment during the process test phase discussed in Section 8. Considerable skill is involved in obtaining a consistently even solder coat. Even electronic firms who routinely dip solder by hand must frequently make soldering iron repairs to their cards. First attempts at dipping cards on the bench were totally unsuccessful. LMSC manufacturing personnel and representatives of several small electronic companies in the area were contacted and their suggestions. though frequently at variance on the fine points of the "art," led to development of a satisfactory technique.

Table 14-2

ACRE TESTS

Test Number	Test Description
1	DC offset of the system was performed. A short was programmed at the input to the test station amplifier. Offset could not be equal to more than 1 count or 7.8 mv.
2	AC offset of the system was performed. Same as dc except the voltage digitizer was programmed to accept an ac voltage.
3	Voltage from a standard cell in the test station was programmed through the amplifier and digitized to check the positive voltage gain. The standard cell had a voltage of 1.01934 v. The amplifier was adjusted for a gain of 4. The output of the amplifier was 4.07736 v. A reading of 522 v would equal 4.0716 (522×7.8), which was 6 mv less than one count on the voltage digitizer.
4	Polarity of the standard cell was reversed. The system was checked for gain on the negative side.
5	The time and frequency digitizer was programmed for self test. The 1-μ sec clock was counted for 512 μ sec.
6	Positive 18 vdc was applied from the test station to the test oscillator. A measurement of this voltage was taken at the oscillator. This ensured the 18 vdc was within 0.05 percent of normal. This also ensured that no variation in circuit parameters was caused by supply voltage variation.
7	DC voltage at the junction of the transistor emitter and resistor I was recorded (no oscillation).
8	DC voltage at the base of the transistor H was recorded (no oscillation).
9	DC voltage at the junction of resistors D and E was shown. The difference between voltages of tests 8 and 9 would be the voltage drop across resistor D (no oscillation).
10	AC voltage at the output of oscillator capacitor J was measured. The negative peak was read on all ac readings.
11	The negative peak voltage, as developed across resistor L through diode K, was measured.
12	AC voltage developed at the emitter of transistor H was recorded. The dc level at this point, as for all ac readings with dc levels, was removed by ACRE.
13	AC voltage developed at the base of transistor H was measured.
14	AC voltage developed at the feedback point was measured. This was the junction of capacitors G and F and resistors D and E.
15	AC voltage developed across the 100-mh coil A was measured.
16	The frequency of oscillations was measured (The ac output).

A holding fixture, tailored to the selected card, was improvised. It consisted of a handle and two pieces of teflon between which the card was clamped. With the solder pot heated to approximately 495° F, the cards were inserted end first at about a 5-deg angle, and then laid flat on the surface of the solder for about 3 sec. This technique worked both on the bench and when cards were dipped in the glove box system.

The flux used was Copper-Brite brand. It did an excellent job of cleaning the copper oxide from the printed circuit when applied in the laboratory atmosphere, in sterile nitrogen, and in sterile air. It sometimes took two or three applications to achieve the same result in the ETO atmosphere, however. This was a minor difficulty and contributed only seconds to the time required to assemble a printed-circuit card in the ETO atmosphere of the MGB.

The first group of cards that was dipped in the MGB gave erratic readings when checked out shortly after dipping. They generally returned to the expected value within a day or two. The problem was traced to flux that continued to adhere to the card after dipping. Alcohol had been used to clean the dipped surface of these cards. It was found that a detergent solution was a much more effective method of removing residual Copper-Brite flux.

On one occasion, the dipping process extended late into the evening and the MGB was not purged before closing the laboratory. The following morning it was noticed that the small circulating fan in the main glove box had a deposit of green verdigris on its blades. It looked, at first glance, as if a mold had formed (which certainly would not have been an endorsement for the sterilizing properties of the MGB atmosphere).

The fan was removed from the MGB and taken to the Palo Alto Materials Sciences Laboratory for analysis. Several types of contamination were found to exist on the fan blade. The first of these contaminants was an oil that was sampled by dusting a small amount of potassium bromide (KBr) on the blade, removing this KBr with a small brush, and forming a pellet. The infrared spectrum on this pellet showed the oil to be a mixture of glycol-like molecules. It is probable that these were derived from the hydrolysis of ethylene oxide.

A second residue, exhibiting a green color, was examined by emission infrared spectroscopy. The infrared examination showed essentially the same oil mixture as the foregoing. Emission spectroscopy showed the major metallic components of the residue to be copper, silver, and tin. Minor components were lead and silicon. There were also trace amounts of cadmium and magnesium. It is most probable that this contamination arose from the absorption of metal vapors originating from the soldering operations carried out in the MGB. The oil already adhering to the fan blades served as an excellent collecting medium.

14.2.2 Sterilization Skips

The first group of 10 cards that were manufactured in the ETO atmosphere of the MGB were cut up and bottled for sterility checking. The first cut was made so as to keep all the capacitors in the circuit on the same half of the card. This same cutting technique was used on all subsequently bottled cards. Each card segment was then bottled in a jar of nutrient, making a total of 20 jars containing 10 printed-circuit cards. Three of these jars produced colonies of B.globigii as determined by turbidity, microscope, and agar plating. All three of the jars contained a segment of printed-circuit card with capacitors in the circuit. The discovery of these contaminated specimens led to a renewed effort to determine the cause of failure-to-kill. These tests and experiments are discussed in Section 15.

14.3 OPERATIONAL COMMENTS

Although each operation performed in the glove box system, including printed-circuit card assembly, was carefully planned prior to its initiation, in almost every case start of the operation led to the need for additional items within the box. These "after-thought" items included a pencil and paper to mark items and the alcohol originally used for circuit-board cleaning.

Charleston Rubber Company Butasol gloves were purchased in the thickest gauge sold (0.03 in.) in order to reduce the possibility of ethylene oxide permeating the rubber. It was thought that the thickness of the gloves would severely limit the operators dexterity. However, the internal pressure in the glove box pressed the gloves firmly against the operator's hand and forearm and made it easier for him to work in the gloves than had been anticipated.

The thin mylar pressure-sensitive tape was stripped from the capacitors in the final groups of cards and bottled separately for sterilization assembly. This operation proved to be the most difficult to perform with the gloves during the program.

The approximate average time to assemble a card on the bench was 35 min. After practice, near the program end, the approximate assembly time in the MGB was 75 min.

14.4 RESULTS

14.4.1 Performance Data Collections

Data were collected from all cards assembled during the program using the ACRE automatic checkout equipment. To analyze the effects of assembly in ETO, sterile air, and sterile nitrogen, however, only data from cards manufactured late in the program were used, as these cards theoretically represented the best products that could be produced by the assembler during the course of the study. How much more proficient the assembler might have become if more time were available is, of course, a matter of conjecture.

The data collected from these performance test cards are presented in Tables 14-4 to 14-13. Table 14-3 indicates from which card the data were collected. Ten tables are required as there are 10 significant data points per card. The data in each column were collected from cards assembled in column (1), the laboratory environment; column (2), ETO; column (3), sterile air; and column (4), sterile nitrogen. The rows represent the time in the card's assembly history at which the data were taken. Row (A) contains the preassembly breadboard data; row (B), the first data taken after assembly; row (C), a second data run after assembly [at least 24 hr after the run in row (B)]; row (D), the first data run after the card was "canned" in its assembly atmosphere; and row (E), the second data run after the card was "canned [at least 24 hr after run (D)].

The tables and graphs in this section are given and plotted in ACRE units. Table 14-14 is a conversion table that permits each point to be converted to its voltage or frequency equivalent.

Table 14-3

PRINTED-CIRCUIT-CARD NUMBER KEY

Event	Card Number Assigned for Assembly in Laboratory Air (1)	Card Number Assigned for Assembly in ETO Atmosphere (2)	Card Number Assigned for Assembly in Sterile Air Atmosphere (3)	Card Number Assigned for Assembly in Sterile Nitrogen Atmosphere (4)
Preassembly Data (Laboratory Environment) (A)	(1) 18 (6) 13 (2) 19 (7) 14 (3) 20 (8) 15 (4) 21 (9) 16 (5) 22 (10) 17	(1) 60 (6) 75 (2) 61 (7) 76 (3) 62 (8) 77 (4) 63 (9) 78 (5) 64 (10) 79	(1) 88 (6) 58 (2) 48 (7) 66 (3) 49 (8) 81 (4) 80 (9) 82 (5) 67 (10) 83	(1) 68 (6) 72 (2) 69 (7) 84 (3) 98 (8) 74 (4) 73 (9) 86 (5) 85 (10) 87
First Data After Assembly (B)	(1) 18 (6) 13 (2) 19 (7) 14 (3) 20 (8) 15 (4) 21 (9) 16 (5) 22 (10) 17	(1) 60 (6) 75 (2) 61 (7) 76 (3) 62 (8) 77 (4) 63 (9) 78 (5) 64 (10) 79	(1) 88 (6) 58 (2) 48 (7) 66 (3) 49 (8) 81 (4) 80 (9) 82 (5) 67 (10) 83	(1) 68 (6) 72 (2) 69 (7) 84 (3) 98 (8) 74 (4) 73 (9) 86 (5) 85 (10) 87
Second Data After Assembly (C)	(1) 18 (6) 13 (2) 19 (7) 14 (3) 20 (8) 15 (4) 21 (9) 16 (5) 22 (10) 17	(1) 60 (6) 75 (2) 61 (7) 76 (3) 62 (8) 77 (4) 63 (9) 78 (5) 64 (10) 79	(1) 88 (6) 58 (2) 48 (7) 66 (3) 49 (8) 81 (4) 80 (9) 82 (5) 67 (10) 83	(1) 68 (6) 72 (2) 69 (7) 84 (3) 98 (8) 74 (4) 73 (9) 86 (5) 85 (10) 87
First Data After Canning (D)	(1) 13 (2) 14 (3) 15 (4) 16 (5) 17	(1) 75 (2) 76 (3) 77 (4) 78 (5) 79	(1) 58 (2) 66 (3) 81 (4) 82 (5) 83	(1) 72 (2) 84 (3) 74 (4) 86 (5) 87
Second Data After Canning (E)	(1) 13 (2) 14 (3) 15 (4) 16 (5) 17	(1) 75 (2) 76 (3) 77 (4) 78 (5) 79	(1) 58 (2) 66 (3) 81 (4) 82 (5) 83	(1) 72 (2) 84 (3) 74 (4) 86 (5) 87

Table 14-4

TEST POINT 7 DATA IN ACRE UNITS

Event	Cards Assembled in Laboratory Air (1)	Cards Assembled in ETO Atmosphere (2)	Cards Assembled in Sterile Air Atmosphere (3)	Cards Assembled in Sterile Nitrogen Atmosphere (4)
Preassembly Data (Laboratory Environment) (A)	(1) 943 (6) 892* (2) 983 (7) 969* (3) 954 (8) 949* (4) 942 (9) 980* (5) 880 (10) 1018* M = 962* R = 126*	(1) 936 (6) 937* (2) 885 (7) 933* (3) 932 (8) 947* (4) 948 (9) 964* (5) 934 (10) 881* M = 932* R = 83*	(1) 963 (6) 992* (2) 970 (7) 955* (3) 938 (8) 956* (4) 978 (9) 952* (5) 911 (10) 918* M = 955* R = 74*	(1) 996 (6) 994* (2) 950 (7) 981* (3) 920 (8) 945* (4) 906 (9) 932* (5) 930 (10) 880* M = 946* R = 114*
First Data After Assembly (B)	(1) 965 (6) 891* (2) 987 (7) 968* (3) 956 (8) 949* (4) 946 (9) 980* (5) 910 (10) 1019* M = 961* R = 128*	(1) 919 (6) 938* (2) 849 (7) 929* (3) 940 (8) 947* (4) 951 (9) 958* (5) 935 (10) 878* M = 930* R = 80*	(1) 959 (6) 997* (2) 971 (7) 949* (3) 938 (8) 958* (4) 980 (9) 953* (5) 911 (10) 919* M = 955* R = 788*	(1) 966 (6) 994* (2) 949 (7) 976* (3) 922 (8) 946* (4) 930 (9) 934* (5) 942 (10) 880* M = 946* R = 114*
Second Data After Assembly (C)	(1) 944 (6) 893* (2) 986 (7) 967* (3) 956 (8) 949* (4) 947 (9) 980* (5) 882 (10) 1019* M = 962* R = 126*	(1) 936 (6) 940* (2) 861 (7) 913* (3) 945 (8) 945* (4) 956 (9) 964* (5) 936 (10) 885* M = 929* R = 79*	(1) 958 (6) 996* (2) 976 (7) 955* (3) 941 (8) 959* (4) 981 (9) 952* (5) 914 (10) 920* M = 956* R = 76*	(1) 982 (6) 995* (2) 950 (7) 982* (3) 921 (8) 946* (4) 918 (9) 934* (5) 934 (10) 882* M = 948* R = 113*
First Data After Canning (D)	(1) 897* (2) 972* (3) 952* (4) 982* (5) 1021* M = 965* R = 124*	(1) 937* (2) 987* (3) 945* (4) 965* (5) 881* M = 943* R = 106*	(1) 1001* (2) 954* (3) 959* (4) 950* (5) 921* M = 957* R = 80*	(1) 995* (2) 962* (3) 942* (4) 939* (5) 873* M = 942* R = 122*
Second Data After Canning (E)	(1) 897* (2) 969* (3) 951* (4) 981* (5) 1020* M = 964* R = 123*	(1) 940* (2) 968* (3) 946* (4) 965* (5) 884* M = 941* R = 84*	(1) 999* (2) 956* (3) 958* (4) 951* (5) 920* M = 957* R = 79*	(1) 995* (2) 969* (3) 939* (4) 939* (5) 876* M = 944* R = 119*

*See Subsection 14.4.3. M = mean R = range

Table 14-5

TEST POINT 8 DATA IN ACRE UNITS

Event	Cards Assembled in Laboratory Air (1)	Cards Assembled in ETO Atmosphere (2)	Cards Assembled in Sterile Air Atmosphere (3)	Cards Assembled in Sterile Nitrogen Atmosphere (4)
Preassembly Data (Laboratory Environment) (A)	(1) 822 (6) 786* (2) 846 (7) 839* (3) 828 (8) 824* (4) 824 (9) 846* (5) 784 (10) 869* M = 833* R = 83*	(1) 817 (6) 816* (2) 763 (7) 815* (3) 817 (8) 825* (4) 823 (9) 835* (5) 814 (10) 782* M = 815* R = 53*	(1) 834 (6) 851* (2) 839 (7) 830* (3) 818 (8) 830* (4) 843 (9) 825* (5) 802 (10) 804* M = 828* R = 47*	(1) 855 (6) 852* (2) 826 (7) 845* (3) 810 (8) 824* (4) 800 (9) 816* (5) 811 (10) 781* M = 824* R = 71*
First Data After Assembly (B)	(1) 843 (6) 786* (2) 850 (7) 839* (3) 830 (8) 824* (4) 826 (9) 847* (5) 809 (10) 869* M = 833* R = 83*	(1) 824 (6) 819* (2) 763 (7) 825* (3) 825 (8) 830* (4) 830 (9) 832* (5) 818 (10) 780* M = 817* R = 52*	(1) 831 (6) 856* (2) 840 (7) 827* (3) 819 (8) 832* (4) 846 (9) 826* (5) 803 (10) 804* M = 829* R = 52*	(1) 853 (6) 853* (2) 825 (7) 843* (3) 810 (8) 825* (4) 821 (9) 818* (5) 824 (10) 780* M = 823* R = 73*
Second Data After Assembly (C)	(1) 822 (6) 787* (2) 849 (7) 837* (3) 830 (8) 824* (4) 826 (9) 846* (5) 785 (10) 869* M = 833* R = 82*	(1) 816 (6) 817* (2) 767 (7) 812* (3) 828 (8) 823* (4) 830 (9) 834* (5) 816 (10) 784* M = 814* R = 50*	(1) 830 (6) 854* (2) 843 (7) 831* (3) 820 (8) 832* (4) 844 (9) 825* (5) 804 (10) 805* M = 829* R = 49*	(1) 849 (6) 854* (2) 825 (7) 845* (3) 810 (8) 825* (4) 810 (9) 817* (5) 814 (10) 781* M = 824* R = 73*
First Data After Canning (D)	(1) 790* (2) 840* (3) 826* (4) 848* (5) 871* M = 835* R = 81*	(1) 816* (2) 870* (3) 823* (4) 836* (5) 782* M = 825* R = 88*	(1) 860* (2) 830* (3) 833* (4) 825* (5) 805* M = 831* R = 55*	(1) 854* (2) 836* (3) 834* (4) 824* (5) 780* M = 826* R = 74*
Second Data After Canning (E)	(1) 789* (2) 838* (3) 826* (4) 847* (5) 869* M = 834* R = 80*	(1) 818* (2) 848* (3) 823* (4) 835* (5) 783* M = 821* R = 65*	(1) 858* (2) 831* (3) 833* (4) 826* (5) 806* M = 831* R = 52*	(1) 853* (2) 842* (3) 829* (4) 823* (5) 779* M = 825* R = 74*

*See Subsection 14.4.3. M = mean R = range

Table 14-6

TEST POINT 9 DATA IN ACRE UNITS

Event	Cards Assembled in Laboratory Air (1)	Cards Assembled in ETO Atmosphere (2)	Cards Assembled in Sterile Air Atmosphere (3)	Cards Assembled in Sterile Nitrogen Atmosphere (4)
Preassembly Data (Laboratory Environment) (A)	(1) 812 (6) 776* (2) 838 (7) 829* (3) 819 (8) 815* (4) 815 (9) 838* (5) 773 (10) 860* M = 824* R = 84*	(1) 806 (6) 805* (2) 751 (7) 805* (3) 807 (8) 815* (4) 812 (9) 825* (5) 804 (10) 771* M = 804* R = 54*	(1) 823 (6) 842* (2) 830 (7) 822* (3) 808 (8) 822* (4) 833 (9) 816* (5) 792 (10) 794* M = 819* R = 48*	(1) 846 (6) 844* (2) 817 (7) 836* (3) 800 (8) 815* (4) 790 (9) 805* (5) 801 (10) 769* M = 814* R = 75*
First Data After Assembly (B)	(1) 833 (6) 775* (2) 841 (7) 830* (3) 820 (8) 815* (4) 817 (9) 838* (5) 798 (10) 861* M = 824* R 86*	(1) 813 (6) 809* (2) 750 (7) 815* (3) 815 (8) 819* (4) 820 (9) 822* (5) 809 (10) 770* M = 807* R = 52*	(1) 821 (6) 846* (2) 831 (7) 818* (3) 809 (8) 823* (4) 837 (9) 817* (5) 794 (10) 795* M = 820* R = 51*	(1) 844 (6) 845* (2) 816 (7) 834* (3) 802 (8) 817* (4) 812 (9) 809* (5) 812 (10) 770* M = 815* R = 75*
Second Data After Assembly (C)	(1) 813 (6) 776* (2) 841 (7) 828* (3) 820 (8) 815* (4) 818 (9) 838* (5) 774 (10) 861* M 824* R 85*	(1) 806 (6) 807* (2) 756 (7) 802* (3) 819 (8) 814* (4) 820 (9) 826* (5) 806 (10) 773* M = 804* R = 53*	(1) 820 (6) 845* (2) 834 (7) 821* (3) 810 (8) 823* (4) 835 (9) 815* (5) 795 (10) 795* M = 820* R = 50*	(1) 841 (6) 845* (2) 816 (7) 836* (3) 801 (8) 817* (4) 800 (9) 807* (5) 804 (10) 770* M = 815* R = 75*
First Data After Canning (D)	(1) 780* (2) 831* (3) 817* (4) 839* (5) 862* M = 826* R 82*	(1) 806* (2) 859* (3) 814* (4) 827* (5) 771* M = 815* R = 88*	(1) 850* (2) 821* (3) 824* (4) 815* (5) 795* M = 821* R = 55*	(1) 845* (2) 827* (3) 824* (4) 814* (5) 769* M = 816* R = 76*
Second Data After Canning (E)	(1) 779* (2) 829* (3) 817* (4) 838* (5) 861* M 825* R = 82*	(1) 807* (2) 837* (3) 814* (4) 826* (5) 773* M = 811* R = 64*	(1) 849* (2) 822* (3) 824* (4) 816* (5) 795* M = 821* R = 54*	(1) 845* (2) 832* (3) 821* (4) 813* (5) 769* M = 816* R = 76*

*See Subsection 14.4.3. M = mean R = range

Table 14-7

TEST POINT 10 DATA IN ACRE UNITS

Event	Cards Assembled in Laboratory Air (1)	Cards Assembled in ETO Atmosphere (2)	Cards Assembled in Sterile Air Atmosphere (3)	Cards Assembled in Sterile Nitrogen Atmosphere (4)
Preassembly Data (Laboratory Environment) (A)	(1) 551 (6) 577* (2) 594 (7) 576* (3) 594 (8) 579* (4) 595 (9) 580* (5) 585 (10) 586* M = 576* R = 9*	(1) 556 (6) 587* (2) 533 (7) 597* (3) 583 (8) 597* (4) 588 (9) 575* (5) 583 (10) 599* M = 591* R = 24*	(1) 668 (6) 544* (2) 573 (7) 576* (3) 592 (8) 587* (4) 492 (9) 585* (5) 547 (10) 580* M = 574* R = 43*	(1) 592 (6) 584* (2) 584 (7) 573* (3) 564 (8) 574* (4) 586 (9) 573* (5) 588 (10) 580* M = 577* R = 11*
First Data After Assembly (B)	(1) 551 (6) 577* (2) 592 (7) 572* (3) 593 (8) 578* (4) 594 (9) 583* (5) 581 (10) 589* M = 580* R = 17*	(1) 536 (6) 572* (2) 576 (7) 577* (3) 573 (8) 590* (4) 566 (9) 570* (5) 569 (10) 593* M = 580* R = 23*	(1) 666 (6) 536* (2) 555 (7) 571* (3) 582 (8) 586* (4) 490 (9) 567* (5) 571 (10) 578* M = 568* R = 50*	(1) 573 (6) 579* (2) 587 (7) 571* (3) 561 (8) 570* (4) 580 (9) 570* (5) 585 (10) 578* M = 574* R = 9*
Second Data After Assembly (C)	(1) 554 (6) 577* (2) 593 (7) 578* (3) 593 (8) 578* (4) 594 (9) 579* (5) 584 (10) 586* M = 580* R = 9*	(1) 549 (6) 573* (2) 580 (7) 577* (3) 572 (8) 593* (4) 566 (9) 570* (5) 569 (10) 593* M = 581* R = 23*	(1) 664 (6) 536* (2) 555 (7) 572* (3) 584 (8) 585* (4) 488 (9) 568* (5) 572 (10) 576* M = 567* R = 49*	(1) 583 (6) 585* (2) 586 (7) 568* (3) 560 (8) 574* (4) 585 (9) 571* (5) 585 (10) 581* M = 576* R = 14*
First Data After Canning (D)	(1) 578* (2) 573* (3) 579* (4) 580* (5) 586* M = 579* R = 13*	(1) 584* (2) 584* (3) 600* (4) 576* (5) 597* M = 588* R = 24*	(1) 529* (2) 572* (3) 583* (4) 565* (5) 575* M = 565* R = 54*	(1) 579* (2) 566* (3) 558* (4) 565* (5) 571* M = 567* R = 21*
Second Data After Canning (E)	(1) 574* (2) 577* (3) 576* (4) 577* (5) 584* M = 578* R = 10*	(1) 574* (2) 582* (3) 593* (4) 570* (5) 592* M = 582* R = 23*	(1) 532* (2) 571* (3) 582* (4) 565* (5) 576* M = 565* R = 50*	(1) 578* (2) 564* (3) 559* (4) 565* (5) 573* M = 568* R = 19*

*See Subsection 14.4.3. M = mean R = range

Table 14-8

TEST POINT 11 DATA IN ACRE UNITS

Event	Cards Assembled in Laboratory Air (1)	Cards Assembled in ETO Atmosphere (2)	Cards Assembled in Sterile Air Atmosphere (3)	Cards Assembled in Sterile Nitrogen Atmosphere (4)
Preassembly Data (Laboratory Environment) (A)	(1) 601 (6) 661* (2) 674 (7) 662* (3) 673 (8) 659* (4) 672 (9) 657* (5) 669 (10) 659* M = 660* R = 5*	(1) 651 (6) 657* (2) 611 (7) 653* (3) 671 (8) 680* (4) 669 (9) 670* (5) 672 (10) 683* M = 669* R = 30*	(1) 656 (6) 636* (2) 672 (7) 668* (3) 680 (8) 675* (4) 568 (9) 768* (5) 657 (10) 665* M = 682* R = 39*	(1) 679 (6) 672* (2) 668 (7) 659* (3) 651 (8) 667* (4) 672 (9) 657* (5) 649 (10) 647* M = 660* R = 25*
First Data After Assembly (B)	(1) 609 (6) 662* (2) 671 (7) 656* (3) 671 (8) 657* (4) 676 (9) 664* (5) 665 (10) 669* M = 662* R = 13*	(1) 617 (6) 657* (2) 654 (7) 653* (3) 656 (8) 674* (4) 647 (9) 657* (5) 655 (10) 668* M = 662* R = 21*	(1) 663 (6) 624* (2) 637 (7) 655* (3) 661 (8) 665* (4) 559 (9) 662* (5) 657 (10) 668* M = 655* R = 44*	(1) 643 (6) 660* (2) 665 (7) 664* (3) 662 (8) 658* (4) 652 (9) 662* (5) 662 (10) 663* M = 661* R = 6*
Second Data After Assembly (C)	(1) 610 (6) 661* (2) 665 (7) 665* (3) 665 (8) 653* (4) 664 (9) 652* (5) 663 (10) 656* M = 657* R = 13*	(1) 637 (6) 661* (2) 661 (7) 659* (3) 658 (8) 669* (4) 651 (9) 658* (5) 658 (10) 669* M = 663* R = 11*	(1) 663 (6) 622* (2) 636 (7) 659* (3) 666 (8) 663* (4) 558 (9) 660* (5) 656 (10) 665* M = 654* R = 43*	(1) 663 (6) 670* (2) 669 (7) 664* (3) 651 (8) 668* (4) 665 (9) 668* (5) 666 (10) 671* M = 668* R = 7*
First Data After Canning (D)	(1) 648* (2) 643* (3) 645* (4) 640* (5) 642* M = 644* R = 8*	(1) 652* (2) 651* (3) 662* (4) 652* (5) 660* M = 655* R = 11*	(1) 602* (2) 648* (3) 648* (4) 644* (5) 650* M = 638* R = 48*	(1) 648* (2) 644* (3) 627* (4) 641* (5) 640* M = 640* R = 21*
Second Data After Canning (E)	(1) 645* (2) 641* (3) 643* (4) 641* (5) 655* M = 645* R = 14*	(1) 648* (2) 649* (3) 656* (4) 646* (5) 665* M = 653* R = 19*	(1) 605* (2) 643* (3) 647* (4) 642* (5) 672* M = 642* R = 67*	(1) 645* (2) 641* (3) 629* (4) 641* (5) 642* M = 640* R = 16*

*See Subsection 14.4.3. M = mean R = range

Table 14-9

TEST POINT 12 DATA IN ACRE UNITS

Event	Cards Assembled in Laboratory Air (1)	Cards Assembled in ETO Atmosphere (2)	Cards Assembled in Sterile Air Atmosphere (3)	Cards Assembled in Sterile Nitrogen Atmosphere (4)
Preassembly Data (Laboratory Environment) (A)	(1) 574 (6) 600* (2) 619 (7) 599* (3) 618 (8) 604* (4) 619 (9) 604* (5) 609 (10) 611* M = 604* R = 12*	(1) 578 (6) 609* (2) 569 (7) 622* (3) 608 (8) 623* (4) 614 (9) 598* (5) 608 (10) 624* M = 615* R = 26*	(1) 707 (6) 565* (2) 596 (7) 600* (3) 617 (8) 613* (4) 511 (9) 606* (5) 553 (10) 604* M = 598* R = 48*	(1) 618 (6) 610* (2) 611 (7) 597* (3) 587 (8) 598* (4) 612 (9) 596* (5) 614 (10) 606* M = 601* R = 14*
First Data After Assembly (B)	(1) 574 (6) 600* (2) 617 (7) 595* (3) 617 (8) 601* (4) 619 (9) 606* (5) 609 (10) 614* M = 603* R = 19*	(1) 559 (6) 596* (2) 600 (7) 605* (3) 598 (8) 616* (4) 590 (9) 595* (5) 594 (10) 621* M = 607* R = 26*	(1) 703 (6) 559* (2) 580 (7) 596* (3) 608 (8) 611* (4) 507 (9) 591* (5) 595 (10) 601* M = 592* R = 20*	(1) 597 (6) 605 (2) 614 (7) 594 (3) 584 (8) 595 (4) 605 (9) 593 (5) 611 (10) 604 M = 598* R = 12*
Second Data After Assembly (C)	(1) 577 (6) 600* (2) 617 (7) 601* (3) 617 (8) 602* (4) 618 (9) 604* (5) 609 (10) 611* M = 604* R = 11*	(1) 571 (6) 597* (2) 604 (7) 603* (3) 596 (8) 619* (4) 591 (9) 594* (5) 593 (10) 618* M = 606* R = 24*	(1) 701 (6) 558* (2) 579 (7) 610* (3) 609 (8) 611* (4) 508 (9) 591* (5) 596 (10) 601* M = 594* R = 53*	(1) 608 (6) 606* (2) 612 (7) 592* (3) 583 (8) 598* (4) 608 (9) 593* (5) 611 (10) 605* M = 599* R = 14*
First Data After Canning (D)	(1) 603* (2) 598* (3) 604* (4) 605* (5) 613* M = 605* R = 15*	(1) 602* (2) 610* (3) 626* (4) 601* (5) 625* M = 613* R = 24*	(1) 552* (2) 596* (3) 608* (4) 588* (5) 599* M = 589* R = 56*	(1) 603* (2) 590* (3) 584* (4) 603* (5) 596* M = 595* R = 19*
Second Data After Canning (E)	(1) 600* (2) 595* (3) 601* (4) 604* (5) 611* M = 602* R = 16*	(1) 598* (2) 607* (3) 620* (4) 596* (5) 619* M = 608* R = 24*	(1) 554* (2) 596* (3) 608* (4) 588* (5) 599* M = 589* R = 54*	(1) 603* (2) 588* (3) 584* (4) 589* (5) 598* M = 592* R = 19*

*See Subsection 14.4.3. M = mean R = range

Table 14-10

TEST POINT 13 DATA IN ACRE UNITS

Event	Cards Assembled in Laboratory Air (1)	Cards Assembled in ETO Atmosphere (2)	Cards Assembled in Sterile Air Atmosphere (3)	Cards Assembled in Sterile Nitrogen Atmosphere (4)
Preassembly Data (Laboratory Environment) (A)	(1) 514 (6) 546* (2) 552 (7) 548* (3) 551 (8) 547* (4) 549 (9) 544* (5) 546 (10) 548* M = 547* R = 4*	(1) 527 (6) 557* (2) 485 (7) 559* (3) 544 (8) 546* (4) 544 (9) 543* (5) 542 (10) 548* M = 551* R = 16*	(1) 553 (6) 513* (2) 548 (7) 546* (3) 552 (8) 544* (4) 555 (9) 558* (5) 488 (10) 550* M = 542* R = 45*	(1) 551 (6) 544* (2) 537 (7) 549* (3) 539 (8) 545* (4) 542 (9) 549* (5) 549 (10) 545* M = 546* R = 5*
First Data After Assembly (B)	(1) 515 (6) 546* (2) 550 (7) 543* (3) 550 (8) 544* (4) 548 (9) 546* (5) 541 (10) 553* M = 546* R = 10*	(1) 510 (6) 565* (2) 537 (7) 556* (3) 541 (8) 541* (4) 539 (9) 541* (5) 539 (10) 544* M = 549* R = 24*	(1) 551 (6) 509* (2) 514 (7) 544* (3) 539 (8) 543* (4) 555 (9) 566* (5) 536 (10) 549* M = 542* R = 57*	(1) 532 (6) 540* (2) 540 (7) 547* (3) 535 (8) 543* (4) 536 (9) 548* (5) 547 (10) 544* M = 544* R = 8*
Second Data After Assembly (C)	(1) 517 (6) 564* (2) 557 (7) 549* (3) 550 (8) 545* (4) 548 (9) 544* (5) 546 (10) 549* M = 550* R = 20*	(1) 520 (6) 566* (2) 538 (7) 540* (3) 539 (8) 544* (4) 560 (9) 544* (5) 538 (10) 542* M = 547* R = 26*	(1) 548 (6) 507* (2) 514 (7) 545* (3) 539 (8) 542* (4) 552 (9) 543* (5) 537 (10) 547* M = 537* R = 40*	(1) 542 (6) 541* (2) 538 (7) 543* (3) 535 (8) 546* (4) 541 (9) 548* (5) 546 (10) 546* M = 545* R = 7*
First Data After Canning (D)	(1) 550* (2) 546* (3) 547* (4) 545* (5) 551* M = 548* R = 6*	(1) 550* (2) 548* (3) 553* (4) 548* (5) 549* M = 550* R = 5*	(1) 501* (2) 544* (3) 540* (4) 540* (5) 545* M = 534* R = 44*	(1) 538* (2) 542* (3) 542* (4) 542* (5) 537* M = 540* R = 5*
Second Data After Canning (E)	(1) 546* (2) 543* (3) 544* (4) 543* (5) 549* M = 545* R = 6*	(1) 544* (2) 542* (3) 544* (4) 541* (5) 543* M = 543* R = 3*	(1) 504* (2) 544* (3) 540* (4) 540* (5) 545* M = 535* R = 41*	(1) 537* (2) 540* (3) 530* (4) 544* (5) 545* M = 539* R = 15*

Table 14-11

TEST POINT 14 DATA IN ACRE UNITS

Event	Cards Assembled in Laboratory Air (1)	Cards Assembled in ETO Atmosphere (2)	Cards Assembled in Sterile Air Atmosphere (3)	Cards Assembled in Sterile Nitrogen Atmosphere (4)
Preassembly Data (Laboratory Environment) (A)	(1) 521 (6) 552* (2) 556 (7) 554* (3) 555 (8) 552* (4) 553 (9) 548* (5) 552 (10) 551* M = 551* R = 6*	(1) 533 (6) 562* (2) 496 (7) 566* (3) 549 (8) 551* (4) 546 (9) 549* (5) 547 (10) 553* M = 556* R = 7*	(1) 545 (6) 519* (2) 555 (7) 551* (3) 552 (8) 548* (4) 564 (9) 563* (5) 496 (10) 555* M = 547* R = 44*	(1) 555 (6) 548* (2) 541 (7) 554* (3) 546 (8) 551* (4) 548 (9) 556* (5) 553 (10) 552* M = 552* R = 8*
First Data After Assembly (B)	(1) 522 (6) 552* (2) 554 (7) 548* (3) 553 (8) 549* (4) 553 (9) 551* (5) 547 (10) 555* M = 551* R = 7*	(1) 530 (6) 546* (2) 542 (7) 544* (3) 547 (8) 545* (4) 544 (9) 546* (5) 544 (10) 549* M = 546* R = 5*	(1) 544 (6) 514* (2) 530 (7) 550* (3) 543 (8) 547* (4) 560 (9) 548* (5) 542 (10) 554* M = 543* R = 40*	(1) 536 (6) 544* (2) 545 (7) 552* (3) 543 (8) 549* (4) 542 (9) 554* (5) 550 (10) 550* M = 550* R = 10*
Second Data After Assembly (C)	(1) 524 (6) 551* (2) 556 (7) 555* (3) 556 (8) 550* (4) 553 (9) 548* (5) 552 (10) 552* M = 551* R = 7*	(1) 526 (6) 548* (2) 543 (7) 545* (3) 545 (8) 547* (4) 544 (9) 546* (5) 544 (10) 546* M = 546* R = 3*	(1) 541 (6) 514* (2) 519 (7) 550* (3) 543 (8) 546* (4) 561 (9) 548* (5) 543 (10) 552* M = 542* R = 38*	(1) 545 (6) 546* (2) 542 (7) 548* (3) 542 (8) 552* (4) 546 (9) 554* (5) 550 (10) 551* M = 550* R = 8*
First Data After Canning (D)	(1) 555* (2) 552* (3) 552* (4) 549* (5) 554* M = 552* R = 6*	(1) 555* (2) 554* (3) 558* (4) 553* (5) 554* M = 555* R = 5*	(1) 508* (2) 550* (3) 544* (4) 545* (5) 551* M = 540* R = 43*	(1) 542* (2) 547* (3) 534* (4) 548* (5) 542* M = 543* R = 14*
Second Data After Canning (E)	(1) 550* (2) 548* (3) 549* (4) 547* (5) 551* M = 549* R = 4*	(1) 549* (2) 548* (3) 548* (4) 546* (5) 548* M = 548* R = 3*	(1) 509* (2) 549* (3) 546* (4) 544* (5) 550* M = 540* R = 41*	(1) 542* (2) 545* (3) 536* (4) 549* (5) 545* M = 543* R = 13*

*See Subsection 14.4.3. M = mean R = range

Table 14-12

TEST POINT 15 DATA IN ACRE UNITS

Event	Cards Assembled in Laboratory Air (1)	Cards Assembled in ETO Atmosphere (2)	Cards Assembled in Sterile Air Atmosphere (3)	Cards Assembled in Sterile Nitrogen Atmosphere (4)
Preassembly Data (Laboratory Environment) (A)	(1) 855 (6) 872* (2) 872 (7) 870* (3) 881 (8) 867* (4) 877 (9) 858* (5) 874 (10) 853* M = 864* R = 19*	(1) 837 (6) 898* (2) 769 (7) 894* (3) 858 (8) 869* (4) 861 (9) 864* (5) 861 (10) 874* M = 880* R = 34*	(1) 730 (6) 834* (2) 869 (7) 757* (3) 871 (8) 784* (4) 862 (9) 893* (5) 864 (10) 878* M = 829* R = 136*	(1) 858 (6) 854* (2) 851 (7) 867* (3) 869 (8) 863* (4) 860 (9) 870* (5) 854 (10) 861* M = 863* R = 16*
First Data After Assembly (B)	(1) 858 (6) 875* (2) 866 (7) 860* (3) 879 (8) 862* (4) 875 (9) 865* (5) 877 (10) 862* M = 865* R = 15*	(1) 811 (6) 868* (2) 864 (7) 857* (3) 854 (8) 861* (4) 856 (9) 861* (5) 859 (10) 870* M = 863* R = 9*	(1) 731 (6) 828* (2) 829 (7) 752* (3) 857 (8) 870* (4) 862 (9) 871* (5) 865 (10) 881* M = 840* R = 129*	(1) 832 (6) 850* (2) 858 (7) 870* (3) 861 (8) 862* (4) 853 (9) 870* (5) 855 (10) 863* M = 863* R = 20*
Second Data After Assembly (C)	(1) 864 (6) 871* (2) 868 (7) 872* (3) 880 (8) 865* (4) 876 (9) 860* (5) 874 (10) 857* M = 865* R = 15*	(1) 827 (6) 870* (2) 866 (7) 860* (3) 853 (8) 865* (4) 856 (9) 859* (5) 858 (10) 863* M = 863* R = 11*	(1) 726 (6) 825* (2) 828 (7) 753* (3) 855 (8) 885* (4) 862 (9) 870* (5) 863 (10) 876* M = 842* R = 132*	(1) 844 (6) 853* (2) 852 (7) 860* (3) 861 (8) 866* (4) 860 (9) 870* (5) 850 (10) 864* M = 863* R = 17*
First Data After Canning (D)	(1) 881* (2) 867* (3) 869* (4) 860* (5) 859* M = 867* R = 22*	(1) 884* (2) 873* (3) 882* (4) 875* (5) 872* M = 878* R = 11*	(1) 817* (2) 874* (3) 858* (4) 864* (5) 875* M = 858* R = 58*	(1) 846* (2) 860* (3) 837* (4) 860* (5) 849* M = 850* R = 23*
Second Data After Canning (E)	(1) 874* (2) 861* (3) 864* (4) 858* (5) 855* M = 862* R = 19*	(1) 869* (2) 861* (3) 865* (4) 859* (5) 862* M = 863* R = 10*	(1) 816* (2) 857* (3) 857* (4) 861* (5) 876* M = 853* R = 60*	(1) 844* (2) 855* (3) 840* (4) 861* (5) 869* M = 854* R = 29*

*See Subsection 14.4.3. M = mean R = range

Table 14-13

TEST POINT 16 DATA IN ACRE UNITS

Event	Cards Assembled in Laboratory Air (1)	Cards Assembled in ETO Atmosphere (2)	Cards Assembled in Sterile Air Atmosphere (3)	Cards Assembled in Sterile Nitrogen Atmosphere (4)
Preassembly Data (Laboratory Environment) (A)	(1) 764 (6) 749* (2) 752 (7) 757* (3) 752 (8) 754* (4) 750 (9) 760* (5) 753 (10) 753* M = 755* R = 11*	(1) 785 (6) 751* (2) 749 (7) 740* (3) 755 (8) 747* (4) 754 (9) 756* (5) 758 (10) 751* M = 749* R = 16*	(1) 752 (6) 797* (2) 750 (7) 749* (3) 750 (8) 750* (4) 748 (9) 750* (5) 722 (10) 744* M = 758* R = 53*	(1) 750 (6) 756* (2) 751 (7) 748* (3) 757 (8) 750* (4) 751 (9) 746* (5) 752 (10) 754* M = 751* R = 10*
First Data After Assembly (B)	(1) 765 (6) 749* (2) 752 (7) 758* (3) 752 (8) 754* (4) 750 (9) 760* (5) 753 (10) 753* M = 755* R = 11*	(1) 789 (6) 752* (2) 752 (7) 742* (3) 755 (8) 748* (4) 756 (9) 757* (5) 759 (10) 750* M = 750* R = 15*	(1) 758 (6) 803* (2) 796 (7) 751* (3) 754 (8) 751* (4) 750 (9) 750* (5) 756 (10) 747* M = 760* R = 56*	(1) 753 (6) 761* (2) 755 (7) 752* (3) 756 (8) 754* (4) 753 (9) 750* (5) 751 (10) 755* M = 754* R = 11*
Second Data After Assembly (C)	(1) 765 (6) 749* (2) 752 (7) 759* (3) 752 (8) 754* (4) 750 (9) 760* (5) 753 (10) 753* M = 755* R = 11*	(1) 789 (6) 750* (2) 751 (7) 740* (3) 755 (8) 748* (4) 756 (9) 756* (5) 760 (10) 750* M = 749* R = 16*	(1) 758 (6) 805* (2) 796 (7) 751* (3) 754 (8) 751* (4) 749 (9) 750* (5) 756 (10) 747* M = 761* R = 58*	(1) 752 (6) 761* (2) 754 (7) 751* (3) 757 (8) 754* (4) 754 (9) 749* (5) 750 (10) 755* M = 754* R = 12*
First Data After Canning (D)	(1) 750* (2) 759* (3) 754* (4) 760* (5) 754* M = 755* R = 10*	(1) 752* (2) 742* (3) 750* (4) 758* (5) 752* M = 751* R = 16*	(1) 805* (2) 752* (3) 753* (4) 752* (5) 748* M = 762* R = 57*	(1) 762* (2) 752* (3) 755* (4) 750* (5) 756* M = 755* R = 12*
Second Data After Canning (E)	(1) 749* (2) 759* (3) 754* (4) 760* (5) 753* M = 755* R = 11*	(1) 752* (2) 742* (3) 749* (4) 758* (5) 752* M = 751* R = 16*	(1) 805* (2) 752* (3) 752* (4) 751* (5) 749* M = 762* R = 56*	(1) 762* (2) 752* (3) 756* (4) 751* (5) 756* M = 755* R = 11*

*See Subsection 14.4.3. M = mean R = range

Table 14-14

CONVERSION TABLE – ACRE UNITS TO ENGINEERING UNITS

Step 1		Step 2		Step 3	
Acre Units	Unscaled Volts	Acre Units	Unscaled Volts	Test Point	Scale Factor
32	0.250	1	0.008	1 dc	0.25
64	0.500	2	0.016	2 ac	0.25
96	0.750	3	0.023	3 dc	0.25
128	1.000	4	0.031	4 dc	0.25
160	1.250	5	0.039	5 f	3.9
192	1.500	6	0.047	6 dc	4.0
224	1.750	7	0.055	7 dc	1.0
256	2.000	8	0.063	8 dc	1.0
288	2.250	9	0.070	9 dc	1.0
320	2.500	10	0.078	10 dc	1.0
352	2.750	11	0.086	11 dc	0.5
384	3.000	12	0.094	12 dc	1.0
416	3.250	13	0.102	13 dc	1.0
448	3.500	14	0.109	14 dc	1.0
480	3.750	15	0.117	15 dc	2.0
512	4.000	16	0.125	16 f	3.9
576	4.500	17	0.133		
608	4.750	18	0.141		
640	5.000	19	0.148		
672	5.250	20	0.156		
704	5.500	21	0.164		
736	5.750	22	0.172		
768	6.000	23	0.180		
800	6.250	24	0.188		
832	6.500	25	0.196		
864	6.750	26	0.204		
896	7.000	27	0.211		
928	7.250	28	0.219		
960	7.500	29	0.227		
992	7.725	30	0.235		
		31	0.243		

Example:
Find mean of cell 1A, Test Point 11. ACRE units are 660.
Step 1: Find next number lower than 660 in ACRE column. This is 640 at 5.000 unscaled volts. 660 - 640 = 20.
Step 2: Find 20 in ACRE units column. Unscaled voltage is 0.156. 5.000 + 0.156 = 5.156.
Step 3: Test Point 1' is at scale factor 0.5. 0.5 × 5.156 = 2.578 vdc.
To obtain frequency for test points 5 and 16 multiply 3.9 cps × ACRE units.

14.4.2 Analysis of Variance

The first analysis of the prodigious amount of data in the foregoing tables was accomplished by an IBM 7094 analysis-of-variance computer program available at LMSC.

The analysis of variance is a statistical technique that permits comparison of the variation in controlled factors with the variation in random uncontrolled factors.

Data should be classified only if the variation of the classification is significantly greater than variation that would occur randomly. In order to obtain a measure of random variation, the LMSC program first computed what is called "within-cell variance" for each cell.* The cell variances were then pooled under the assumption that the random variance within cells was comparable in size. This pooling was done to obtain as many degrees of freedom as possible for the measure of random variance.

The 7094 computer program is capable of handling up to six criteria of classification and will analyze data if the cells contain equal or proportional amounts of data. In this analysis, only two classification criteria were available, the atmosphere in which the card was assembled and tested and the succession of testing, which was a rough measure of time in the atmosphere. The computer program allocated the total variance of all of the data to each of the criteria of classification and also determined if there was any interaction between criteria of classification.

The variance allotted to each criterion of classification was then compared with the random variance. This ratio is called the F ratio. If F is equal to 1, the variance associated with the criteria of classification is no larger than random variation, and the effect of the criteria of classification is meaningless.

---

*The term "cell" denotes all data within a square of Tables 14-4 to 14-13.

If the F ratio is smaller or larger than 1, it is necessary to use a table of F ratios to determine if the departure from 1 is significant enough not to have happened because of chance alone. If the difference is large enough so that it would not occur oftener than 5 percent of the time as a result of chance, then the conclusion can be drawn that the criterion of classification is meaningful and contributed to the variation. If the observed F ratio is larger than the 1 percent probability point, the same conclusion is reached but with greater confidence in the results of the experiment.

It was necessary to run an analysis of variance for each data point, i.e., one for each of Tables 14-4 through 14-13. The first row of these tables had to be disregarded in the analysis, as all values obtained in this row were obtained in the laboratory environment. The results of the analysis of variance are presented in Table 14-15.

The F ratios obtained (reading horizontally across rows of the tables) for test points 7, 11, 13, 14, and 15 exceed the corresponding 5 percent probability points. The ratio for test point 15 exceeds the 1 percent probability point.

Only one F ratio for test succession (reading vertically down columns of the table) exceeds the 5 percent probability point. This is for test point 11. This is the only variance analysis evidence that succession of testing affected the data.

Without further analysis, the results of the variance analysis appear to indicate that changing the assembly atmosphere did affect the components on the card as represented by test points 7, 11, 13, 14, and 15.

The validity of this assumption is placed in doubt by the abnormally low F ratios for succession of testing at test points 10, 12, and 16. The reciprocals of the F ratios for these three test points are shown. One of them is well above the corresponding 1 percent point and the other two are well above the corresponding 5 percent probability point. This throws serious doubt upon the measure of random variance. Theoretically, nothing can vary significantly less than random variance due to chance alone.

Table 14-15

ANALYSIS OF VARIANCE OF TEST DATA

Test Point Number	Mean Squares			Observed F Ratio		Expected F Ratio		Observed 1/F	Expected 1/F	
	Atmosphere (Horiz.)[a]	Test Succession (Vert.)[b]	Within Cells	Atmosphere (Horiz.)[a]	Test Succession (Vert.)[b]	5 Percent Prob. Point	1 Percent Prob. Point	Test Succession (Vert.)[b]	5 Percent Prob. Point	1 Percent Prob. Point
7	4383.00	387.33	1241.01	3.531	0.312	2.70	3.98	–	–	–
8	1241.67	209.67	540.58	2.296	0.387	2.70	3.98	–	–	–
9	1373.33	199.67	565.03	2.430	0.353	2.70	3.98	–	–	–
10	785.83	33.17	455.21	1.726	0.072	2.70	3.98	13.725	8.68	26.60
11	951.83	1023.17	329.91	2.885	3.101	2.70	3.98	–	–	–
12	719.00	34.33	547.04	1.314	0.062	2.70	3.98	15.933	8.68	26.60
13	371.00	27.17	130.73	2.837	0.207	2.70	3.98	–	–	–
14	330,33	22.83	84.62	3.903	0.269	2.70	3.98	–	–	–
15	3645.33	460.00	605.55	6.019	0.759	2.70	3.98	–	–	–
16	406.67	5.00	158.12	2.571	0.031	2.70	3.98	31.625	8.68	26.60
17	1522.50	–	568.79	2.676	–	3.98	7.01	–	–	–

[a] Values obtained while reading horizontally across Tables 14-4 through 14-13.
[b] Values obtained while reading vertically down Tables 14-4 through 14-13.

When a source of variance is encountered that is significantly smaller than random variance, it indicates that the measure used for random variance was inflated due to the presence of some unknown factor.

An examination of the data in Tables 14-4 through 14-13, when referred back to the key (Table 14-3) that identifies from which card the data were obtained, shows that the variation between cards in a cell at most data points was significant and that, even near the end of the program when these data were obtained, it was impossible to make one circuit that performed exactly like the last regardless of where it was assembled. This was not due to the atmospheres in which the cards were made, but was due to the sensitive nature of the circuit and the wide beta spread on the transistor in the circuit. Unfortunately the printed-circuit cards could not be made to perform in a sufficiently uniform manner to render the variance analysis wholly meaningful. As data from the same cards appear in cells arranged vertically in the matrices (successive stages of testing), this analysis has the greatest significance.

14.4.3 Analysis of Means and Ranges

A second analysis of the data in Tables 14-4 through 14-13 was performed. Data obtained from the same cards in each vertical column are indicated by asterisks in the tables. The mean and the range of each of these readings were computed, and are also indicated by asterisks. The means of each test point for the cards assembled in each atmosphere were then plotted against the different stages of testing. In this analysis, row (A) was meaningful as the data in these cells represented the breadboard or initial conditions. These plots are shown in Figs. 14-6 through 14-8. When the data are presented in this manner it can be seen that the same general data pattern is followed regardless of the atmosphere in which the cards were assembled.

The ranges of readings in cells exhibit no pattern that is unique and repeatable. The largest ranges occur in cells containing readings from cards assembled in the laboratory (128) and in sterile air (132). When the ranges are computed in vertical columns, it is found that the readings from cell to cell are reasonably constant, which again

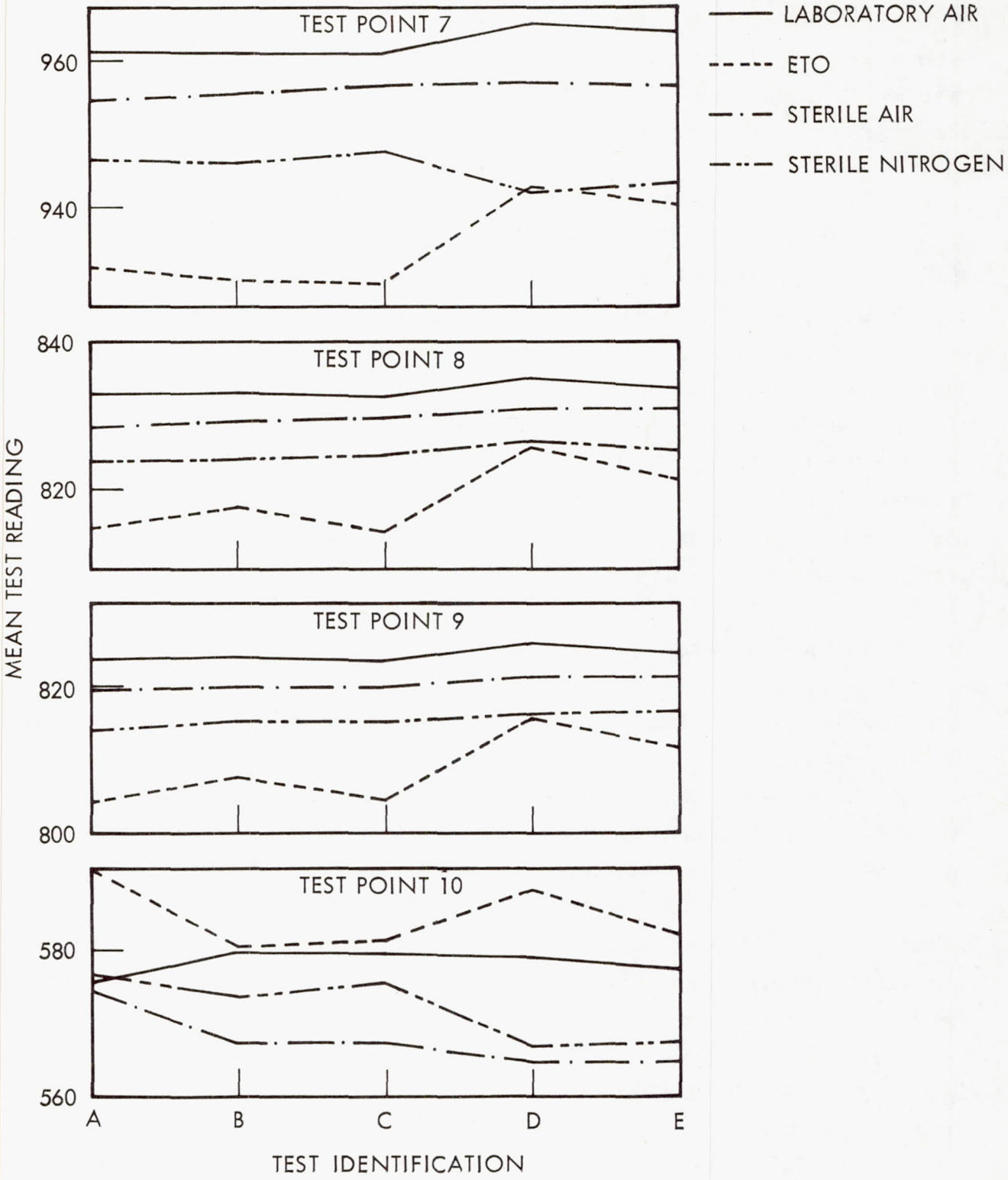


Fig. 14-6 Test Value Comparison, Test Points 7 to 10

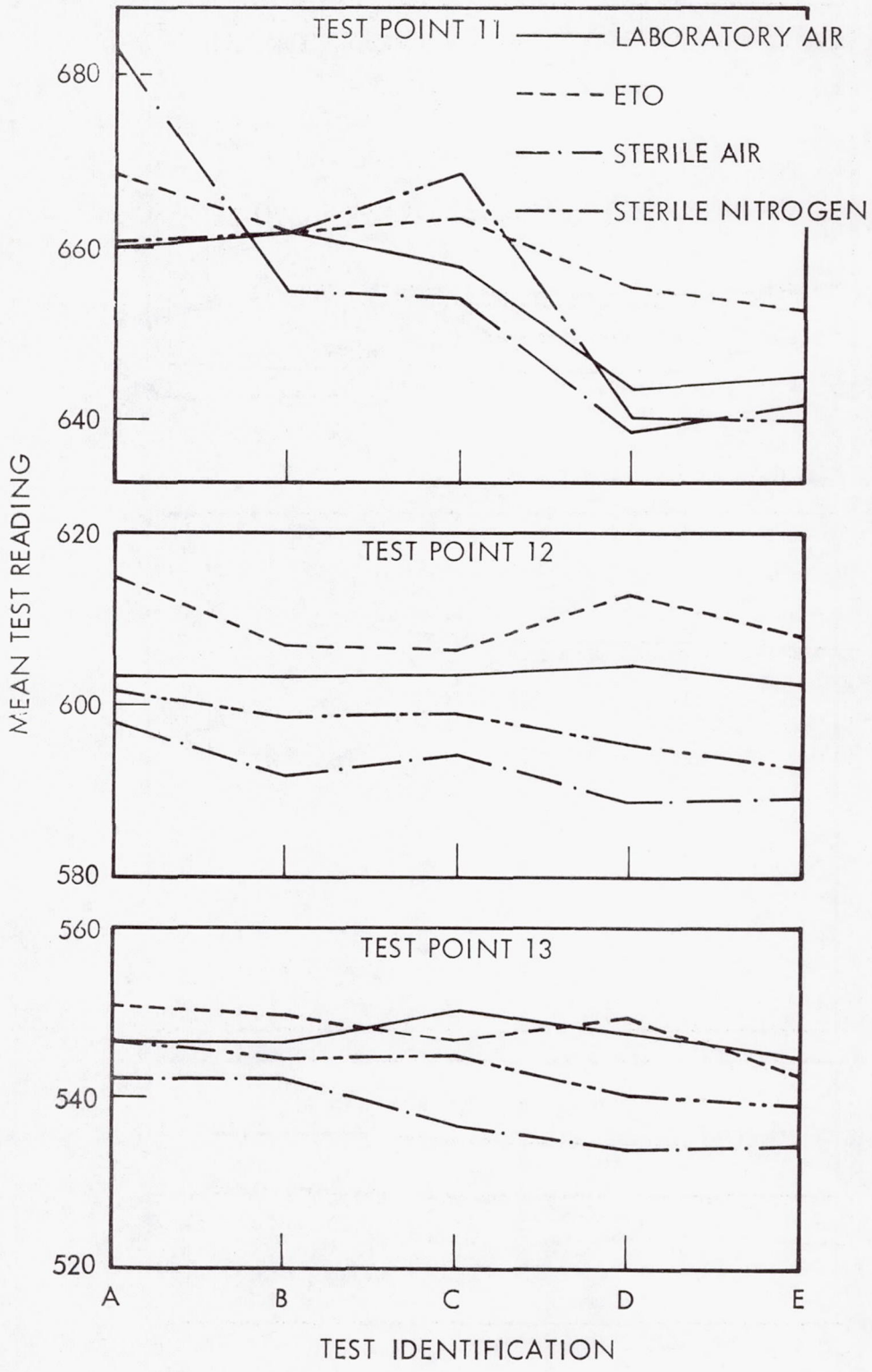


Fig. 14-7 Test Value Comparison, Test Points 11 to 13

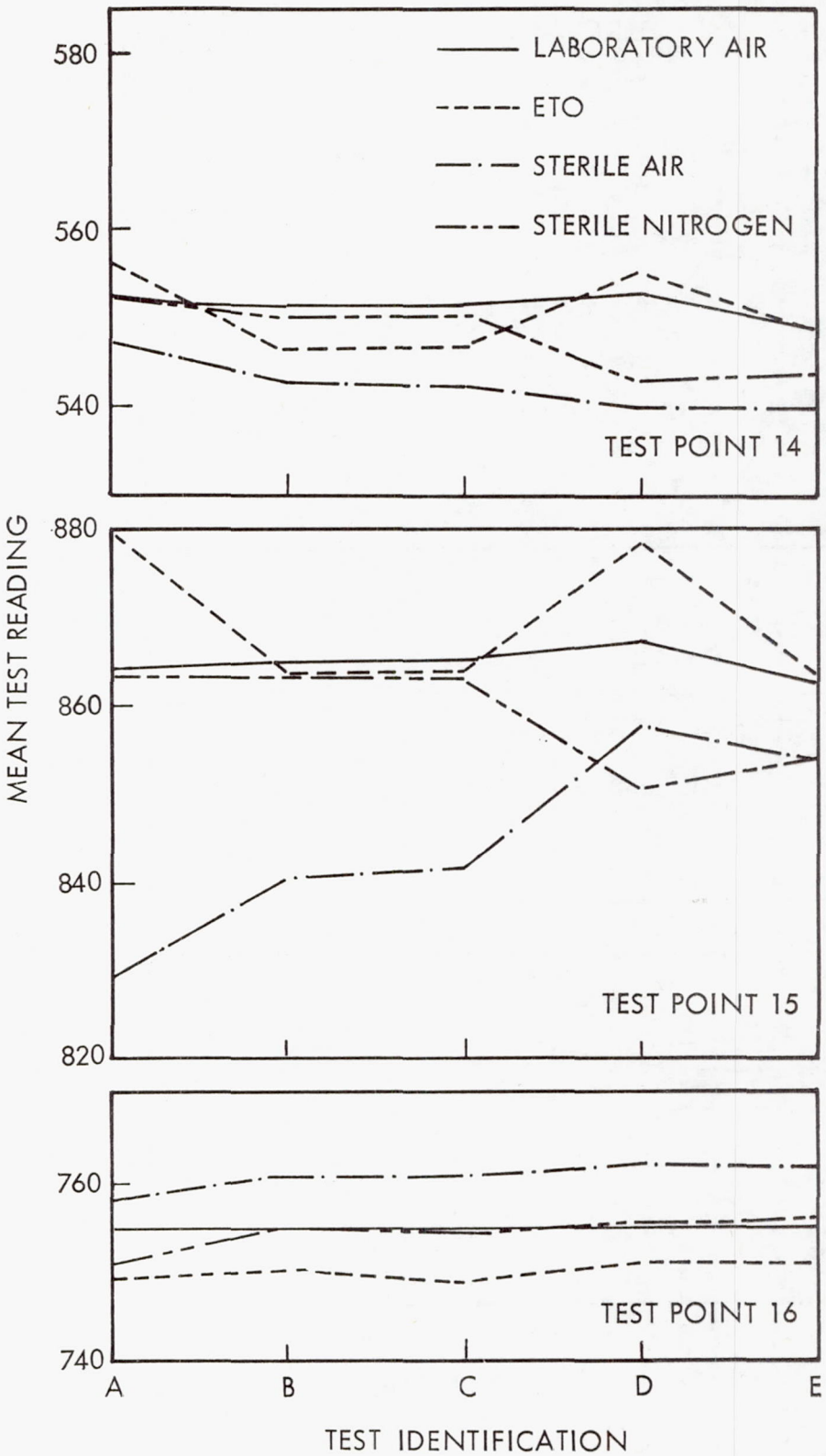


Fig. 14-8 Test Value Comparison, Test Points 14 to 16

indicates that each circuit tends to be unique but does not change appreciably from its breadboard configuration on the bench (in laboratory atmosphere) to its manufactured condition in the assembly atmosphere.

From these data, analyses, and the absence of any catastrophic or repetitive circuit failures during the course of the study, it can be concluded that assembly of printed-circuit card units and similar operations are feasible in ETO, sterile air, and sterile nitrogen following a soak period in ETO.

14.4.4 Sterility Testing of Assembled Circuits

The results of the sterilization assays performed on printed-circuit cards assembled during the program are summarized in Table 14-16. The first group of 10 cards assembled in ETO and bottled in 20 jars of nutrient resulted in 3 nonsterile jars. These contaminated items triggered investigations into the cause of contamination that continued until the end of the program. The results of these investigations are presented in Section 15.

As the program completion date was fast approaching when the contamination was discovered, it was not possible to interrupt the circuit assembly to solve the failure-to-kill problem. Card assembly, therefore, continued in parallel with the investigations discussed in Section 15. For this reason the nonsterile specimens indicated in Table 14-16 were anticipated. In an attempt to localize this expected contamination, the mylar tape was stripped from the capacitors when subsequent circuits were cut for bottling. The tapes from each circuit card were placed in separate jars and incubated. Detailed data on these cards are presented in Appendix A, pages A-10 through A-18.

The tapes from the capacitors contributed to contamination of 11 of the 23 jars (following the initial ETO group). Eight of the stripped capacitors were contaminated, and four of the printed-circuit card segments containing the resistors, the transistor, and the diode were not sterile. It was quite difficult to strip the pressure-sensitive tape from the capacitors in the glove box, and it is quite possible that fragments of the mylar and some of the adhesive remained on the body of the capacitor.

Table 14-16

SUMMARY OF STERILITY TEST RESULTS

Card Description	No. of Cards	No. of Jars	No. of Toxic Jars	No. of Contam. Jars	Remarks*
First ETO Assembled	10	20	4	3	3-C
Second ETO Assembled	5	15	0	5	3-T, 1-C, 1-R
ETO Canned	5	15	0	4	1-T, 2-C, 1-R
Sterile-Air Assembled	6	18	0	2	2-T
Sterile-Air Canned	5	15	1	8	3-T, 4-C, 1-R
Sterile-Nitrogen Assembled	6	17	0	1	1-T
Sterile-Nitrogen Canned	5	14	0	3	1-T, 1-C, 1-R
Bench Assembled	5	15	0	0	Not Inoculated
Bench Canned	5	15	3	0	Not Inoculated

*C – Portion of card with capacitors and coil.
T – Mylar tapes removed from capacitors.
R – Portion of card with resistors, transistor, and diode.

The bench assembled circuits, five of which were canned, were heat sterilized but not inoculated with B. globigii spores prior to being placed in the MGB. Inoculation was omitted in order to get a feel for the sterilizing properties of the atmosphere on the types of organisms that these components would pick up while being handled in a normal electronic-circuit manufacturing environment. As seen from Table 14-16, none of these items was contaminated. The canned bench items were exposed to the ETO atmosphere for less than 1/2 hr.

Section 15
CONTAMINATION INVESTIGATION

15.1 INITIAL INVESTIGATION AND RESULTS

Failure to sterilize occurred first during the process test phase of the program. These skips, which are discussed in Sections 7, 8, 10, and 12 of this report, occasioned several previously unplanned experiments and resulted in modifications to the glove box systems.

Contamination of the process test items could have resulted from several causes, including contamination in the small glove box during bottling, contamination of the culture media during preparation, or failure to kill the organisms during ethylene oxide exposure in the main glove box. It was, therefore, necessary to explore each alternative. At the time the contamination was discovered, nine card groups which were intended for assembly, dip soldering, and checkout in the next phase of the program were undergoing sterilization in the main glove box. Individual components from several of these card groups were bottled in culture media and checked for sterility. They had been in the box for five days. Some were passed into the small glove box, and some were bottled in the main glove box. Results of this test are summarized in the following table:

No. of Components	Type Component	Where Bottled	No. not Sterile
5	Capacitors	Main Glove Box	3
7	Capacitors	Small Glove Box	2
3	Resistors	Main Glove Box	0
7	Resistors	Small Glove Box	0
1	Coil	Main Glove Box	1

No. of Components	Type Component	Where Bottled	No. not Sterile
2	Coil	Small Glove Box	1
1	Transistor	Main Glove Box	Toxic
2	Transistor	Small Glove Box	1
1	Diode	Main Glove Box	0
2	Diode	Small Glove Box	0
1	Printed Circuit Card	Main Glove Box	1
3	Printed Circuit Cards	Small Glove Box	1

Those items bottled in the main glove box were placed in jars of sterile broth as rapidly as possible, thus minimizing the time period during which the jars were open. To test this procedure, two spore strips containing 100 B. globigii spores were inserted in jars of culture media in the main glove box. Growth occurred in one of these jars. Detailed results of the biological assays made on these components are found in Appendix A, pages A-19 through A-21.

In another test, air being supplied to the small glove box was directed into an open jar of culture media. The jar was then sealed, passed out of the glove box system, and incubated. No growth occurred in this jar. One jar of media was opened in the small glove box and a finger of one glove dipped in the broth. This was repeated in the main glove box. No growth occurred in either of these jars. Detailed data on these tests appears on pages A-19 and A-21 of Appendix A. To check on the sterility of the culture media being supplied, 15 jars were placed in the incubator for 24 hours and checked for growth. No growth occurred in any of these jars. Contamination in all tests conducted was B. globigii. No extraneous contamination was found in any tests.

The above tests indicated that the cause of contamination was most probably failure to kill the B. globigii spores in the main glove box and that ability of the spores to survive was related to type of component, probably to some characteristic of the components surfaces. The spores seemed hardest to kill when deposited on the capacitors.

In an attempt to check this theory, five inoculated capacitors were placed in an ethylene oxide sterilizer at the Palo Alto biology laboratory and were exposed to the gas for 48 hours. All five capacitors were found to be contaminated following this exposure. As the Palo Alto unit has no provision for water vapor addition this test could not conclusively prove the resistance of B. globigii spores deposited on capacitors. However, had this test experiment killed the B. globigii spores, it would have indicated that procedures being used at the Sunnyvale sterilization laboratory were at fault.

15.1.1 Control of Main Glove Box Humidity

After localizing the problem area to the main glove box sterilization chamber, measures were taken to determine the probable cause of failure-to-kill, and to develop solutions which would re-establish confidence in the sterilization procedures.

Since the start of the program, a Mine Safety Appliance infrared water vapor indicator had been showing relative humidities in the main glove box of from 55 percent to 80 percent; the readings were most frequently in the vicinity of 60 percent. Efforts to reduce the humidity included placing silica gel inside the chamber, and frequent displacement of the atmosphere with fresh, dry, ethylene oxide/Freon 12 gas mixture. The silica gel remained in the atmosphere for two weeks during which time no appreciable reduction in main glove box relative humidity was noted, according to the Mine Safety Appliance instrument.

Following discovery of the non-sterile test specimens, efforts to determine the true relative humidity present in the atmosphere were intensified. A supply of gas was taken from the glove box and analyzed for water vapor content using a chromatograph. This analysis indicated 15 percent relative humidity. A hand aspirating psychrometer was then inserted in the glove box and also gave a reading of 15 percent relative humidity. As a further check, a wet and dry bulb thermometer was used which again indi-

cated 15 percent relative humidity. At this point a beaker of distilled water was inserted in the glove box and sufficient water was evaporated to raise the humidity to 45 percent as indicated by the wet and dry bulb instrument. An electrical humidity indicator, supplied by JPL, was then used to monitor the main glove box atmosphere. This instrument gave an indication of 48 percent, confirming the wet and dry bulb instrument's reading within 5 percent. The wet and dry bulb instrument was used for the remainder of the program to monitor relative humidity. No additional problems were experienced from this time on in maintaining relative humidity between 20 percent and 40 percent.

15.1.2 Main Glove Box Gas Circulation

In addition to low relative humidity, it was possible that poor circulation of the gas mixture within the glove box might be contributing to the failure-to-kill. Two methods of improving circulation within the box were incorporated. Four nylon cords were strung parallel to the longitudinal axis of the main glove box, approximately 18 in. above the floor. Subsequent specimens being sterilized were suspended from these cords. Knots at 2 in. intervals in the cords keep adjacent specimens separated. The gas mixture thus had free access to all surfaces of the specimens being treated. To further improve gas circulation, a small muffin fan of the type used to circulate air in electronic cabinets was installed in the glove box. The fan kept the gas in motion throughout the box and eliminated any tendency for it to stagnate or pocket. The fan is shown in Fig. 15-1.

15.1.3 Flask Ethylene Oxide Concentration

Jet Propulsion Laboratory personnel suggested by technical memorandum that a possible cause of sterilization skips might be a reduced concentration of ethylene oxide in the gas mixture used. Accordingly, several analyses of the Matheson Company ethylene oxide/Freon 12 mixture used during the program were made. Both infrared spectrometry and gas chromatography techniques were used in an attempt to determine the exact composition of the gas. The experience proved to be an education in the problems of obtaining valid samples of volatile gas mixtures.

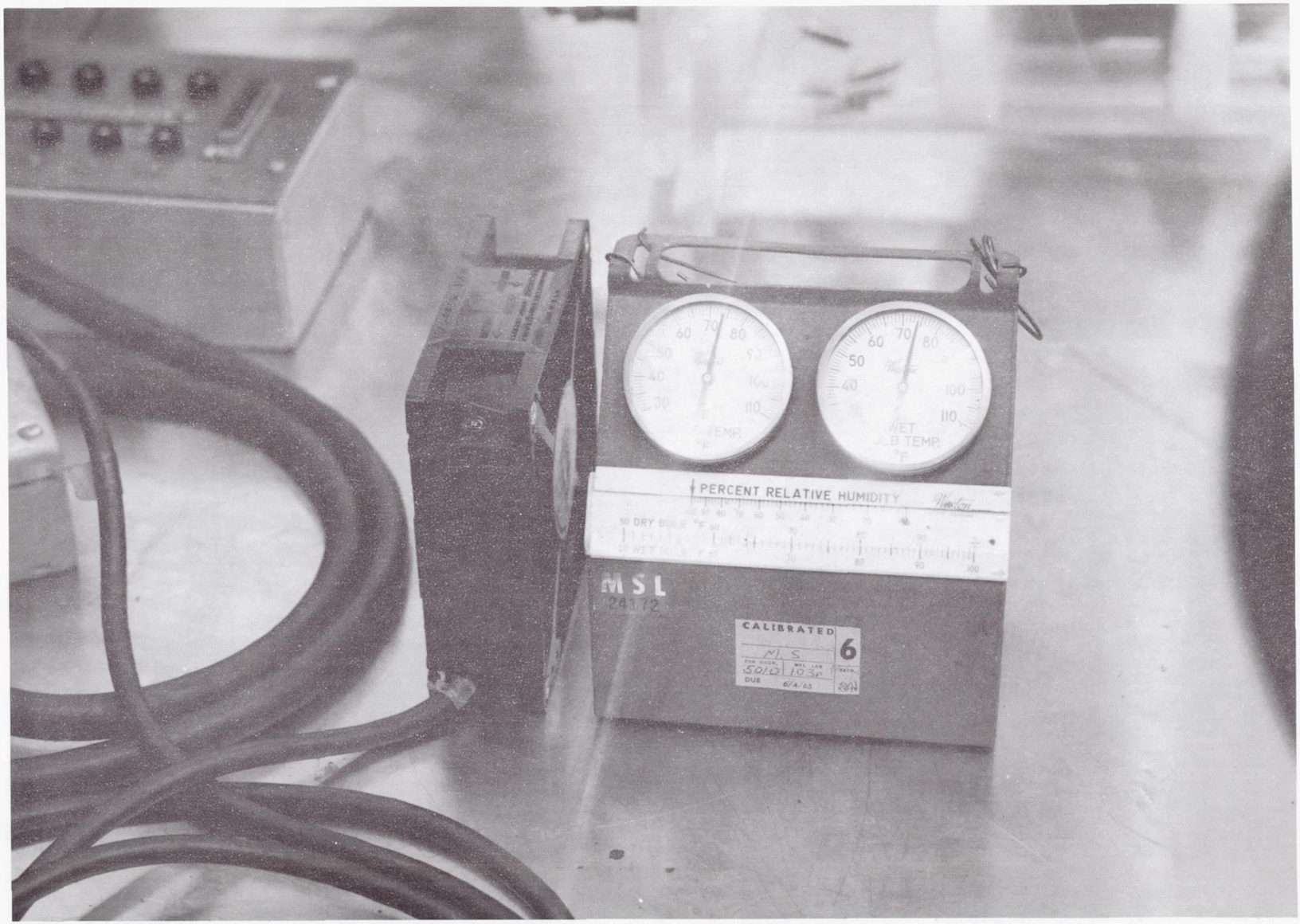


Fig. 15-1 Glove Box Circulating Fan (Shown With Humidity Indicator)

The initial samples were taken directly from the heated discharge line from the flask. These were gaseous samples and invariably indicated a low concentration of ethylene oxide; about 21 percent by volume (27 percent by volume is the correct ratio assuming a perfect gas relationship). At this time, an order was immediately placed for the JPL-recommended Pennsylvania Engineering Company "Penn Gas" ethylene oxide/ Freon 12 gas mixture.

Conversations with Matheson Company and LMSC chemists, however, brought out the importance of obtaining a liquid phase sample for a true analysis. The vapor pressure of the mixture, about 70 psi, made this more difficult. A small pressure cylinder (150 cc) was procured and a liquid sample obtained by flowing through the cylinder until liquid appeared at the outlet. Samples were again run on the Matheson flask using both the infrared spectrometer and gas chromatograph. To make a true measurement, it is necessary to vaporize the entire liquid sample and measure all the gas produced. In practice this is difficult even for a 150 cc sample of the liquid. Measurements were made of the initial boil-off from the flask using the infrared technique. A 10-cm-path cell loaded to approximately 100 mm of Hg pressure was utilized for these determinations. Table 15-1 presents the data obtained during this study.

The Freon 12 concentrations in the infrared cell were probably reliable. This molecule has a very excellent analytical absorption band in the 665 cm^{-1} region. The ethylene oxide analytical band at 3,068 cm^{-1} is not optimum in shape, but is the only available band which will allow reasonable analytical data.

Table 15-1

ANALYSIS OF GAS MIXTURES

Sample	Concentration of Gas (Percent by Volume) F-12	ETO
1	78.6	21.4
	77.0	21.4
	77.9	20.9
2	75.5	25.4
3	73.1	24.3

Chromatographic measurements were made of the final vaporized gas. This instrument gave a concentration of 29.5 percent.

The measurements, according to Lockheed chemists,do not show marked discrepancies from the expected value of 27 percent ethylene oxide concentration and indicate that the Matheson Company gas is within specifications. They also imply that the most accurate way of obtaining a proper gas mixture is by weighing each constituent prior to mixing. A proper mixture, therefore, is dependent on the accuracy of the weighing techniques and equipment and, of course, on the integrity of the vendor.

15.1.4 Sterile Air and N_2 Sampling

In order to further remove any question as to whether contamination was being introduced to the small glove box, Jet Propulsion Laboratory personnel suggested by technical memorandum that Lockheed install a filter system in the sterile air and nitrogen supply line to the small glove box which would permit a sterility determination to be made through culturing of the filter material. A filter holder containing sterilized cotton was installed at the inlet to the small glove box. The holder was accessible from inside the box so that the cotton could be changed aseptically using the gloves. The filter cotton was removed three times and was cultured in trypticase soy broth during the program. No growth occurred. The broth was then inoculated with 10^2 spores and confirmed to be non-toxic. Detailed data on these tests can be found in Appendix A, pages A-22 and A-32.

15.1.5 Sterilization Time Period Test

Following the incorporation of the above modifications, and after obtaining control of glove box humidity, a sterilization time period determination was run using inoculated capacitors. (The original time period determination test was run using resistors.) Ten capacitors were exposed to the mixture in the main glove box for 48 hours, and 10 for 72 hours. Spore strips which consisted of small pieces of filter paper on which

at least 10^6 spores deposited were inserted with the capacitors into the ETO atmosphere. These strips were used as control items throughout the remainder of the program. In both the 48 and 72 hour cases, all capacitors and spore strips were sterile. In subsequent tests, all specimens were exposed to the ETO for a sterilization period of at least 72 hours.

The detailed results of these tests are presented in Appendix A, page A-4. The outcome of these experiments gave the impression that the measures to improve the sterilizing effectiveness of the main glove box atmosphere had been successful. The program as originally planned was resumed.

15.2 SUBSEQUENT CONTAMINATION INVESTIGATIONS AND RESULTS

Following assembly and checkout, the first group of 10 printed circuit cards assembled in ETO in the main glove box were cut up and bottled for a sterility determination. Half of each card was placed in a jar of nutrient. Of these 20 jars, three were found to be contaminated. The results of the assay on these jars is found in Appendix A, pages A-8 and A-9. These results renewed the efforts to determine the cause of failure-to-kill. These experiments are discussed in subsequent paragraphs. As sterilization skip tests continued until the end of the program, it was necessary to conduct them in parallel with printed circuit card assembly.

As had been the case when the first instance of contamination was discovered, there were several circuit card groups which had been in the main glove box system for several days. The contaminated jars all contained the portion of the printed circuit cards having the capacitors and also the coil. Five capacitors and four coils from the available groups were immediately bottled. These had been exposed to the gas for about 60 hours. One of the capacitors was contaminated, and the remaining components were sterile. See Appendix A, page A-22. From this test and past experience, it was concluded that the source of contamination on the printed circuit cards was most likely the capacitor.

15.2.1 Capacitor Covering Tests

The unique feature of the Sprague Vitamin Q capacitor used on the program was a strip of pressure sensitive mylar tape wound around the body of the component (Fig. 15-2).

The tape was removed from six capacitors which had been in the main glove box for 144 hours. The tape was bottled in two jars and each "bare" capacitor in a jar. These capacitors were all sterile. One of the jars containing the mylar coverings (3) was contaminated (Appendix A, page A-23).

Another experiment examined the sterilization obtained when the mylar tape was removed from the capacitor before dipping in the aqueous spore suspension (Appendix A, page A-24). Five capacitors coated with the original mylar tape were sterile following a 72-hour ETO exposure. One of five capacitors without the mylar tape was contaminated. The test spore strip was sterile. Some of the adhesive might have adhered to this capacitor when the tape was removed.

The exact composition of the capacitor covers was determined by contacting the vendor and, in parallel, by absorption spectroscopy. While this was being accomplished, a test was run using a material which was immediately available and appeared the same as the covering material, Scotch brand tape.

Five aluminum plates covered with Scotch tape and five which were not were heat sterilized, inoculated, and placed in the main glove box where they remained for 216 hours. All but one of the coated plates were contaminated. Two of the five uncoated plates were contaminated (Appendix A page A-29).

The above tests presented strong evidence that the coating on the capacitor contributes to the difficulty experienced in sterilizing this item.

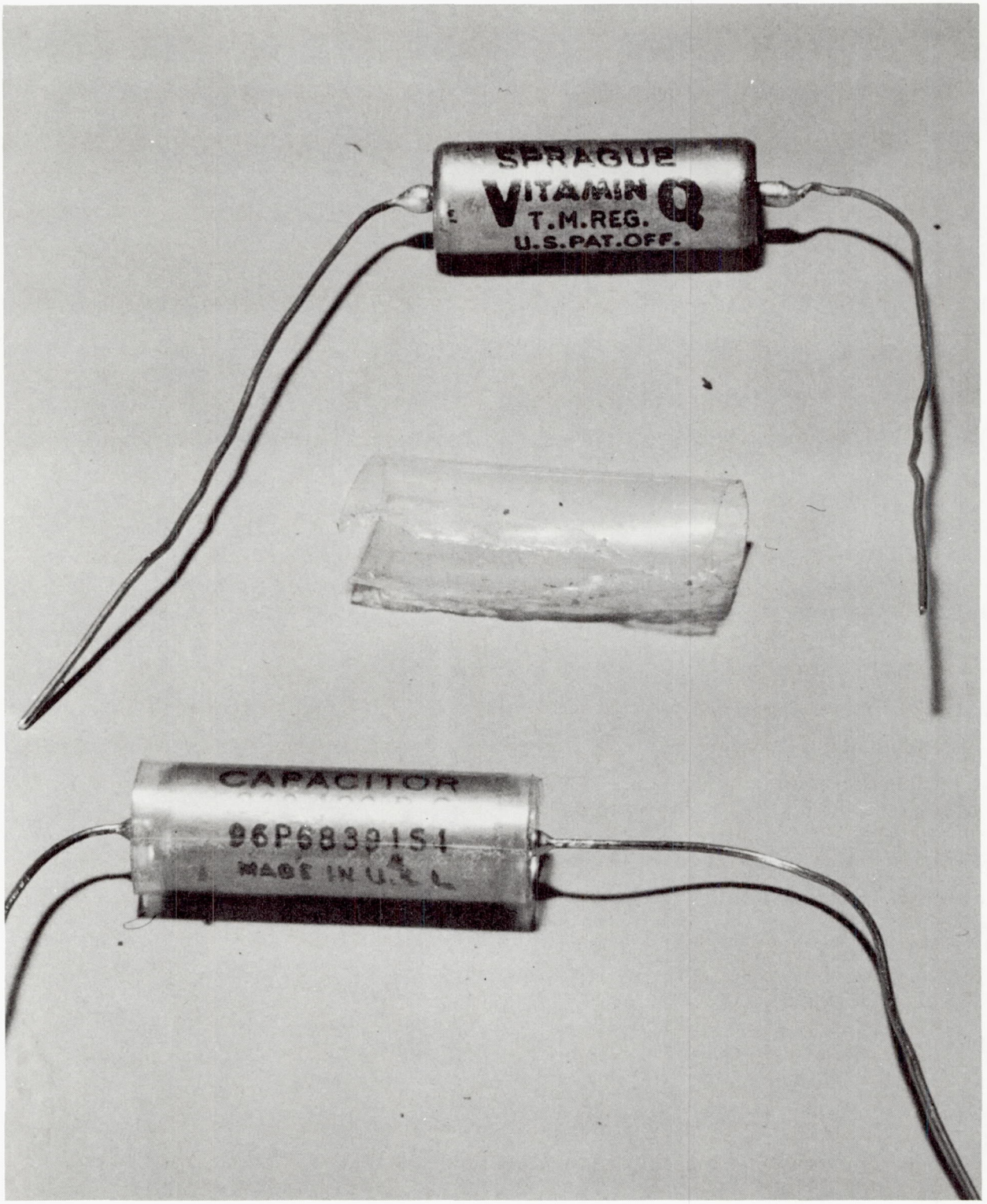


Fig. 15-2 Sprague Vitamin Q Capacitors, Showing Mylar Cover

15.2.2 Effect of Spore Deposition Technique

A test was run to determine if the method by which spores were inoculated contributed to their resistance to ethylene oxide. In the inoculation method used during the program, components were contaminated by total immersion in an aqueous solution of spores. During the dipping process the spore might be carried into areas where the ethylene oxide gas could not penetrate or some unknown mechanism might cause it to be protected.

Another method of inoculation with B. globigii spores is the use of acetone as the carrier fluid.

The following experiment was performed: Group A – Vitamin Q capacitors were dipped in an aqueous spore suspension; Group B – Vitamin Q capacitors were dipped in acetone spore suspension; Group C – A concentrated acetone spore suspension was carefully placed on the outer tape coating of the Vitamin Q capacitors. All capacitors were sterilized in the sterile assembly box with ethylene oxide and tested for sterility by the standard procedure. Four exposure times to ethylene oxide were used – 24 hours, 48 hours, 120 hours, and 144 hours (Appendix A, pages A-25, A-26, A-27, and A-28).

Of the water dipped capacitors, 18 of 20 test capacitors were non-sterile. The acetone dipped capacitors showed some improvement in obtaining sterilization. Six out of 20 acetone dipped capacitors were non-sterile. The 20 capacitors inoculated with a drop of acetone spore suspension gave four non-sterile samples.

A filter paper spore strip and a glass slide containing 10^6 spores deposited from an acetone suspension were sterile. Increasing the exposure time to ethylene oxide beyond 24 hours has no effect on improving sterilization. Improved sterilization was obtained with those capacitors contaminated with acetone treated spore suspensions.

The results are summarized in Table 15-2.

Table 15-2

EFFECT OF SPORE DEPOSITION ON STERILIZATION

Treatment	ETO Exposure Time	Sterile	Non-Sterile	Total Sterile	Total Non-Sterile
Water dipped	24	0	5		
Water dipped	48	2	3		
Water dipped	120	0	5		
Water dipped	144	0	5	2	18
Acetone dipped	24	3	2		
Acetone dipped	48	3	2		
Acetone dipped	120	3	2		
Acetone dipped	144	5	0	14	6
Acetone spotted	24	5	0		
Acetone spotted	48	4	1		
Acetone spotted	120	3	2		
Acetone spotted	144	4	1	16	4

15.2.3 Experiments With 100-Percent Ethylene Oxide

The effect of increasing the concentration of ethylene oxide to 100 percent was studied in three experiments. Capacitors were mounted in a portable ethylene oxide sterilizer consisting of a sealed plastic cylinder (Fig. 15-3). The cylinder was evacuated (21 in. of Hg) and charged with pure ethylene oxide. Distilled water (0.02 ml) was injected into the incoming gas stream. The cylinder was then passed into the main glove box. Three tests were run with exposure of 6.5 hours, 54 hours, and 60 hours. Less than half of the capacitors were sterilized in 60 hours; all capacitors exposed in the other two tests

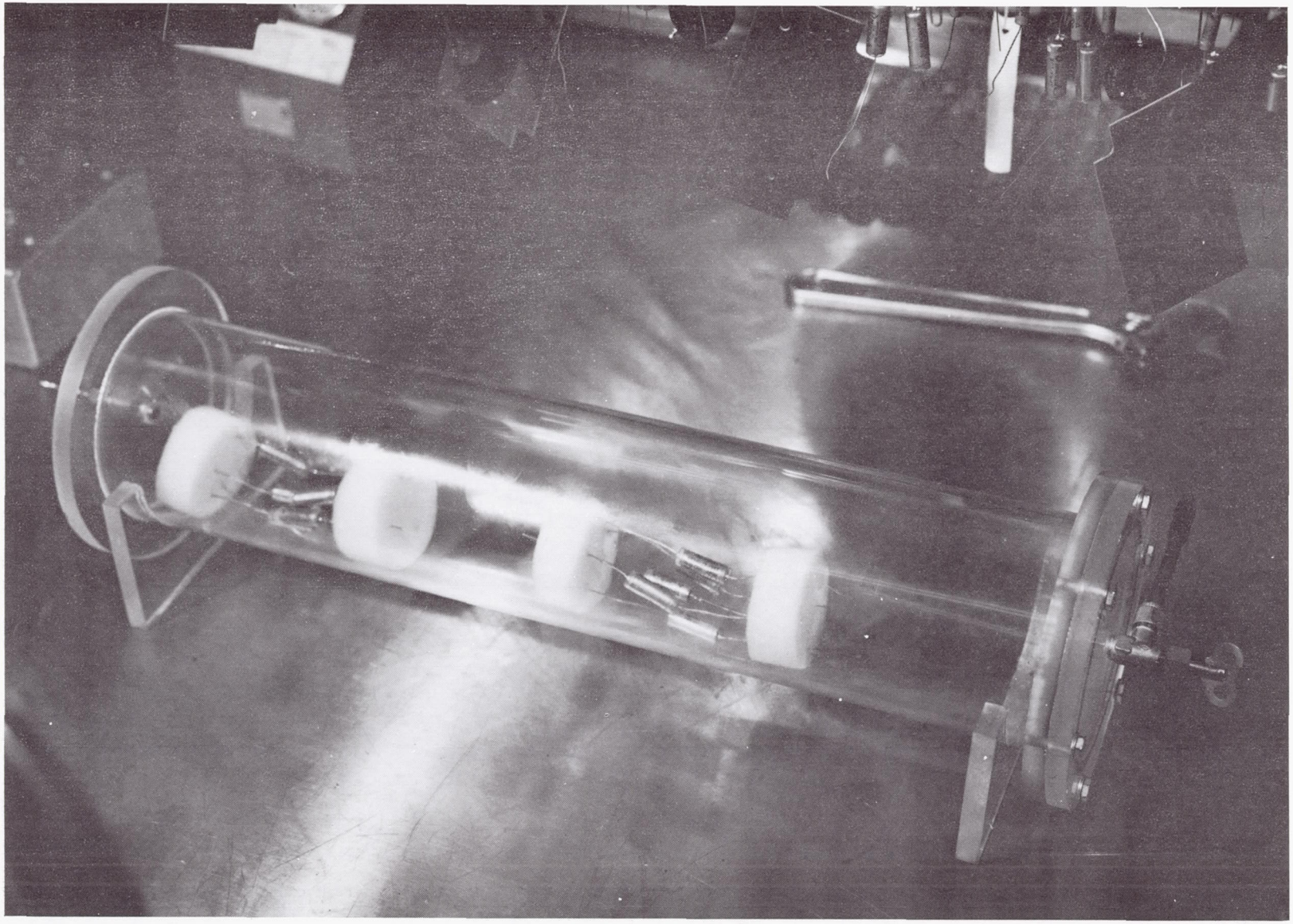


Fig. 15-3 Portable Ethylene Oxide Sterilizer, Shown in Main Glove Box

were contaminated. The results are presented in Table 15-3. Detailed information is presented in Appendix A, pages A-31, A-32, and A-33.

Table 15-3

STERILIZATION IN 100-PERCENT ETHYLENE OXIDE

Exposure Time	Sterile	Non-Sterile
6.5	0	10
54	0	10
60	5	6

These tests proved how difficult the Sprague Vitamin Q1 capacitor is to sterilize using ethylene oxide gas. At the present time no definite explanation can be made for this high resistance to the gas.

15.2.4 Effect of High Humidity and Rehydration

The ability to kill with ethylene oxide has been demonstrated by many researchers to be related to the humidity of the gas. At relative humidities below 20 percent, kill has been shown to drop off sharply. A less gradual reduction in kill has been demonstrated at relative humidities above 50 percent. Recent investigations have indicated that complete wetting of the item to be sterilized greatly increases the probability of achieving sterility.

A test was conducted to check this theory using the capacitor, and to test if any difference in ability to sterilize could be detected between components inoculated in the normal manner and those inoculated by aqueous suspension and not dried. Twenty capacitors were dipped and dried and then immersed in distilled water immediately before being placed in the ETO atmosphere. An additional 20 were inoculated and immediately placed in ETO without drying. Ten of each group of 20 were placed in the portable ETO

sterilizer, which had been used in the 100 percent ethylene oxide test. The tube was charged with ETO rather than pure ethylene oxide for this test. The only non-sterile capacitors, as shown in Table 15-4, were two which had not been dried after dipping and which had been sterilized in the portable sterilizer. Detailed results of this test are found on pages A-33 and A-34 of Appendix A. The results of this test did not provide evidence to support or to weaken the hydration and rehydration theories.

Table 15-4

HUMIDITY AND REHYDRATION TEST

Description	Number Sterile	Number Not Sterile	Number Total Parts
Non-dried Sterilized in main glove box	10	0	10
Dried and rehydrated Sterilized in main glove box	10	0	10
Non-dried Sterilized in Portable sterilizer	8	2	10
Dried and rehydrated Sterilized in portable sterilizer	10	0	10
Total	38	2	40

Appendix A
STERILE ASSEMBLY TECHNIQUES BACTERIOLOGY REPORT

The bacteriology reports in Appendix A were compiled by the LMSC biologists who performed the sterilization assays on all specimens they received from the Sunnyvale Sterilization Laboratory during the study.

The number of the jar containing nutrient and test sample is listed in the first column of the tables constituting the appendix. The next column describes the study phase during which that sample was bottled for assay. The next column describes the visual appearance of that jar following the 7-day incubation period. In the next column, the results of microscopic examination of a sample of the jar's contents are presented. If viable organisms were observed through the microscope, they generally are described as "rods" (the characteristic shape of B. Globigii). The "Check for Growth by Transfer" heading covers two subcolumns, the first showing the result of a tripticase soy agar plate check of the jar contents and the second a check made by transferring a portion of the contents to an additional tube of broth for turbidimetric analysis. "Neg" entries in both these subcolumns indicate that the jar was sterile or toxic.

The toxicity was determined by inoculating the jar with 10^2 organisms if the microscopic, agar plate, and turbidimetric tests were negative. If no growth occurred, the nutrient was considered toxic; this is noted in the column headed "Degree of Component Toxicity." The last two columns, "Sterile Yes/No" and "Remarks," are self-explanatory.

Table A-1

STERILE ASSEMBLY TECHNIQUES BACTERIOLOGY REPORT
– TEST NO. 1 STERILIZATION TIME PERIOD –

Jar No.	Assembly Step and Description (Sterilization Time Period)	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
HA	I R C Resistors							
1	48 hr sterilization – turned	Clear	Neg	Neg	Neg	Not toxic to 10^2 inoculum per 15 ml	Yes	
2	↓	↓	↓	↓	↓	↓	↓	
3								
4								
5								
HB								
1	48 hr sterilization – not turned	Clear	Neg	Neg	Neg	Not toxic to 10^2 inoculum per 15 ml	Yes	
2	↓	↓	↓	↓	↓	↓	↓	
3								
4								
5								
HA								
1	28 hr sterilization – turned	Clear	Neg	Neg	Neg	Not toxic to 10^2 inoculum per 15 ml	Yes	
2	↓	↓	↓	↓	↓	↓	↓	
3								
4								
5								
HB								
1	28 hr sterilization – not turned	Clear	Neg	Neg	Neg	Not toxic to 10^2 inoculum per 15 ml	Yes	
2	↓	↓	↓	↓	↓	↓	↓	
3								
4								
5								
HA								
1	24 hr sterilization – turned	Clear, no change	Neg	Neg	Neg	Not toxic to 10^2 inoculum per 15 ml	Yes	
2	↓	↓	↓	↓	↓	↓	↓	
3								
4								
5								

Table A-1 (Cont.)

Jar No.	Assembly Step and Description (Sterilization Time Period)	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
HB	I R C Resistors							
1	24 hr sterilization – not turned	Clear, no change	Neg	Neg	Neg	Not toxic to 10^2 inoculum per 15 ml	Yes	
2	↓	Clear, no change	Neg	Neg	Neg	↓	Yes	
3		Clear, no change	Neg	Neg	Neg		Yes	
4		Clear, no change	Neg	Neg	Neg		Yes	
5		Clear, no change	Neg	Neg	Neg		Yes	
HA								
1	15 hr sterilization – turned	Turbid, growth present	Rods	B. globigii	Growth	Not toxic to 10^2 inoculum per 15 ml	No	Two of this group not sterile
2	↓	↓	Rods	B. globigii	Growth	↓	No	
3		Clear, no change	Neg	Neg	Neg		Yes	
4		Clear, no change	Neg	Neg	Neg		Yes	
5		Clear, no change	Neg	Neg	Neg		Yes	
HB								
1	15 hr sterilization – not turned	Clear, no change	Neg	Neg	Neg	Not toxic to 10^2 inoculum per 15 ml	Yes	One of this group not sterile
2	↓	Turbid, growth present	Rods	B. globigii	Growth	↓	No	
3		Clear, no change	Neg	Neg	Neg		Yes	
4		Clear, no change	Neg	Neg	Neg		Yes	
5		Clear, no change	Neg	Neg	Neg		Yes	
HA								
1	4 hr sterilization – turned	Turbid, growth present	Rods	B. globigii	Turbid	Not toxic to 10^2 inoculum per 15 ml	No	One of this group not sterile
2	↓	Clear, no change	Neg	Neg	Neg, clear	↓	Yes	
3		Clear, no change	Neg	Neg	Neg, clear		Yes	
4		Clear, no change	Neg	Neg	Neg clear		Yes	
5		Clear, no change	Neg	Neg	Neg, clear		Yes	
HB								
1	4 hr sterilization – not turned	Turbid, growth present	Rods	B. globigii	Turbid	Not toxic to 10^2 inoculum per 15 ml	No	One of this group not sterile
2	↓	Clear	Neg	Neg	Neg	↓	Yes	
3		Clear	Neg	Neg	Neg		Yes	
4		Clear	Neg	Neg	Neg		Yes	
5		Clear	Neg	Neg	Neg		Yes	

Table A-2

STERILE ASSEMBLY TECHNIQUES BACTERIOLOGY REPORT
– TEST NO. 2 STERILIZATION TIME PERIOD –

Jar No.	Assembly Step and Description (Sterilization Time Period)	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
T001	Capacitors in for 48 hr	Clear, no color change	Neg	Neg	Neg	Not toxic to inoculum 10^2	Yes	
T002	↓	↓	Neg	Neg	Neg	↓	Yes	
T003			Neg	Neg	Neg		Yes	
T004			Neg	Neg	Neg		Yes	
T005			Neg	Neg	Neg		Yes	
T006			Neg	Neg	Neg		Yes	
T007			Neg	Neg	Neg		Yes	
T008			Neg	Neg	Neg		Yes	
T009			Neg	Neg	Neg		Yes	
T010	↓	↓	Neg	Neg	Neg	↓	Yes	
Spore Strip SS-1		Clear, no color change	Neg	Neg	Neg	Not toxic to inoculum 10^2	Yes	
T011	Capacitors in for 72 hr	Clear, no color change	Neg	Neg	Neg	Not toxic to inoculum 10^2	Yes	
T012	↓	↓	Neg	Neg	Neg		Yes	
T013		Moderate turbidity	Neg	Neg	Neg		Yes	
T014		Clear, no color change	Neg	Neg	Neg		Yes	
T015			Neg	Neg	Neg		Yes	
T016			Neg	Neg	Neg		Yes	
T017			Neg	Neg	Neg		Yes	
T018			Neg	Neg	Neg		Yes	
T019			Neg	Neg	Neg		Yes	
T020	↓	↓	Neg	Neg	Neg	↓	Yes	
Spore Strip SS-2		Clear, no color change	Neg	Neg	Neg	Not toxic to inoculum 10^2	Yes	

Table A-3

STERILE ASSEMBLY TECHNIQUES BACTERIOLOGY REPORT – PROCESS TESTS –

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
1	Process test specimens – nut bolt assembly	Clear broth with heavy sediment in bottom of jar	Inconclusive	Neg	Neg	Toxic to inoculum 10^6	Inconclusive	
2	↓	↓	Inconclusive	Neg	Neg	↓	Inconclusive	
3			Inconclusive	Neg	Neg		Inconclusive	
4		Clear, no sediment	Neg	Neg	Neg		Inconclusive	
5		Clear broth with heavy sediment in bottom of jar	Inconclusive	Neg	Neg		Inconclusive	Sterilized 5 days
6	Resistor to printed circuit card. Sterility check prior to dip solder.	Turbid, contaminated	Rods	B. globigii	Turbid, growth	–	No	Three of this group of four not sterile
7	↓	Turbid, contaminated	Numerous rods	B. globigii	Turbid, growth	–	No	
8		Clear, green	Neg	Neg	Clear	Toxic to 10^6	Undetermin-able	Toxic
9		Turbid, contaminated	Rods	B. globigii	Turbid, growth	–	No	Sterilized 4 days
10	Dip soldering a component to a printed circuit card. Glove box assembled, sterility test specimens.	Turbid, no color change	Neg	Neg	Neg	Not toxic to inoculum of 10^6 per 100 ml	Yes	This group of four samples appear sterile. Good growth obtained with inoculum of 10^6.
11	↓	↓	Neg	Neg	Neg	↓	Yes	
12			Neg	Neg	Neg		Yes	
13			Neg	Neg	Neg		Yes	
14			Neg	Neg	Neg		Yes	Sterilized 4 days
16	Dip soldering a component to a printed circuit card. Glove box assembled test specimens.	Clear, no color change	Neg	Neg	Neg	Not toxic to inoculum of 10^6 per 100 ml	Yes	Growth obtained with inoculum of 10^6
17	↓	Extremely turbid	Neg	Neg	Neg	↓	Yes	
18		Extremely turbid	Neg	Neg	Neg		Yes	
23		Extremely turbid	Neg	Neg	Neg	Not toxic to inoculum of 10^6 per 100 ml	Yes	
24		Extremely turbid	Neg	Neg	Neg		Yes	Sterilized 10 days

Table A-3 (Cont.)

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
SW 1	Process specimen test. Staking to a card component in glove box. Eight cards in bottle.	Darkened, brownish black color with 3 + turbidity.	Inconclusive Heavy debris, stain OK	Neg	Neg	Toxic to 10^2 inoculum	Inconclusive	Toxic
SW 2	Same, jar contained 7 cards.	Darkened, brownish black color with 2 + turbidity.	Rods	B. globigii present	Growth	–	No	Sterilized 5 days
25	I R C Resistor to small piece of printed circuit card with epoxy.	Slight discoloration	Rods	B. globigii	Growth	–	No	
27	↓	Medium green color slight turbidity	Neg	Neg	Neg	–	Yes	
28	↓	Medium green color slight turbidity	Neg	Neg	Neg	Not toxic to inoculum 10^2	Yes	Sterilized 21 days
SCU 2	Resistor-wire to component. Sterility check specimens prior to soldering.	Clear, no color change	Neg	Neg	Neg	Not toxic to inoculum of 10^6	Yes	
43	↓	Slightly turbid	Rods	B. globigii	Turbid	–	No	
22	↓	Clear	Neg	Neg	Neg	Not toxic to inoculum 10^6	Yes	
28	↓	Clear	Neg	Neg	Neg		Yes	Sterilized 5 days
9	*Soldering a wire to a component.* Glove box assembled sterility test specimens.	*Broth clear with* heavy sediment in bottom of jar.	*Neg*	*Neg*	*Neg*	*Not toxic to* inoculum 10^6	Yes	
23	↓	↓	Neg	Neg	Neg	↓	Yes	
31	↓	↓	Neg	Neg	Neg	↓	Yes	
32	↓	↓	Neg	Neg	Neg	↓	Yes	
47	↓	↓	Neg	Neg	Neg	↓	Yes	Sterilized 5 days
11-31	Process test specimens metal plate to a printed circuit card. Box assembled sterility test specimens.	Turbidity 4 +	Heavy debris. Stain OK	Neg	Neg	Toxic to 10^2 inoculum	Inconclusive	Toxic
12-32	↓	Gray color, flocculent precipitate	↓	Neg	Neg	Toxic to 10^2	Inconclusive	Toxic
13-33	↓	*Turbidity 2 +*	↓	Neg	Neg	Toxic to 10^2	Inconclusive	Toxic
14-34	↓	Slight gray flocculent precipitate	↓	Neg	Neg	Toxic to 10^2	Inconclusive	Toxic
15-35	↓	Dark green color with flocculent precipitate	↓	Neg	Neg	Toxic to 10^2	Inconclusive	Sterilized 5 days

Table A-3 (Cont.)

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
PS 1	Five metal plates. Unbonded sterility check.	Dark gray black color. Turbidity 2 +	Inconclusive debris	Neg	Neg	Toxic to 10^2	Inconclusive	Toxic
PS 2	Five circuit boards. Unbonded sterility check.	Dark green color	Neg	Neg	Neg	Toxic to 10^2	Inconclusive	Toxic Sterilized 5 days
49-50	Process test specimens. Metal plate to metal plate. Box assembly sterility test.	Clear	Neg	Neg	Neg	Toxic to 10^2	Inconclusive	Toxic
51-52		Turbidity 3 +	Neg	Neg	Neg	Toxic to 10^2	Inconclusive	Toxic
53-54		Turbidity 4 +	Rods	B. globigii	Growth	–	No	
55-56		Turbidity 1 +	Neg	Neg	Neg	Toxic to 10^2	Inconclusive	Toxic
47-48		Turbidity 6 +	Rods	B. globigii	Growth	–	No	Sterilized 5 days

Table A-4

STERILE ASSEMBLY TECHNIQUES BACTERIOLOGY REPORT
– TEST NO. 1 CARDS ASSEMBLED IN MGB IN ETO –

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
29-1	Circuit board, 4 IRC, 1 transistor	Sl cloudy	Neg	Neg	Neg	10^2 inoculum not toxic	Yes	All sterilized for 5 days
-2	Circuit board, 4 Vit Q, 1 coil	Sl cloudy	Neg	Neg	Neg	10^2 inoculum not toxic	Yes	
30-1	Board, 4 IRC, 1 transistor	Clear, no color change	Neg	Neg	Neg	10^2 inoculum not toxic	Yes	
-2	Board, 3 Vit Q Cap. 1 coil	Turbid, gray green	Neg	Neg	Neg	Toxic to 10^2 inoculum	Inconclusive	Toxic
31-1	Board, 4 IRC, 1 transistor	Sl cloudy	Neg, debris	Neg	Neg	Toxic to 10^2 inoculum	Inconclusive	Toxic
-2	Board, 5 Vit Q, 1 coil	Sl cloudy	Neg, debris	Neg	Neg	Not toxic 10^2 inoculum	Yes	
32-1	Board, 6 IRC, 1 transistor diode	Sl cloudy greenish color	Neg	Neg	Neg	Not toxic 10^2 inoculum	Yes	
-2	Board, 4 Vit Q cap. 1 coil	Sl cloudy greenish color	Neg	Neg	Neg	Toxic to 10^2 inoculum	Inconclusive	Toxic
33-1	Board, 5 IRC 1 transistor, diode	Sl cloudy	Neg	Neg	Neg	Not toxic	Yes	
-2	Board, 4 Vit Q cap 1 coil	Cloudy, orange	Rods	B. Globigii	Growth	–	No	
34-1	1 IRC, 1 transistor 1 diode, Board	Clear	Neg	Neg	Neg	Not toxic 10^2 inoculum	Yes	
-2	Board, 4 Vit Q 1 coil	Sl green, clear	Neg	Neg	Neg	Toxic 10^2 inoculum	Inconclusive	Toxic
35-1	Board, 5 IRC, diode, 1 transistor	Green, sl cloudy	Neg	Neg	Neg	Not toxic 10^2	Yes	
-2	Board, 4 Vit Q 1 coil	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	

Table A-4 (Cont.)

Jar No.	Assembly Step and Description	Culture Media Apperance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
36-1	Board, 5 IRC diode transistor	Clear	Neg	Neg	Neg	Not toxic 10^2 inoculum	Yes	
-2	Board, 4 Vit Q, coil	Clear	Neg	Neg	Neg	Not toxic 10^2 inoculum	Yes	
37-1	Board, 5 IRC, diode transistor	Turbid, sl orange color	Neg	Neg	Neg	Not toxic 10^2 inoculum	Yes	
-2	Board, 4 Vit Q, coil	Turbid, orange	Rods	B. globigii	Pos	–	No	
39-1	Board, 5 IRC diode, transistor	Sl cloudy	Neg	Neg	Neg	Not toxic 10^2 inoculum	Yes	
-2	Board, 4 Vit Q, coil	Turbid, orange	Rods	B. globigii	Growth	–	No	
SS01	Spore Strip	Flocculant precipitate (probably dissolving filter paper)	Neg	Neg	Neg	Not toxic 10^2 inoculum	Yes	Sterilized 5 days

Table A-5

STERILE ASSEMBLY TECHNIQUES BACTERIOLOGY REPORT
– TEST NO. 2 CARDS ASSEMBLED IN MGB IN ETO –

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
60-1	Card and I R C resistors	Turbid. sl green	Neg	Neg	Neg	Not toxic 10^2	Yes	All sterilized for 20 days
60-2	Card and Vit Q, coil	Turbid, sl green	Neg	Neg	Neg	Not toxic 10^2	Yes	
60-3	Tape from Vit Q cap.	Turbid, orange	Rods	B. globigii	Growth	–	No	
61-1	Card and I R C resistors	Sl green and turbid	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
61-2	Card and Vit Q, coil	Sl green, clear	Neg	Neg	Neg	↓	Yes	
61-3	Tape from Vit Q cap.	Clear	Neg	Neg	Neg	↓	Yes	
62-1	Card and I R C resistors	Sl green and turbid	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
62-2	Card and Vit Q, coil	Sl green and turbid	Neg	Neg	Neg	↓	Yes	
62-3	Tape from Vit Q cap.	Turbid, orange	Rods	B. globigii	Growth	–	No	
63-1	Card and I R C resistors	Orange, turbid	Rods	B. globigii	Growth	–	No	
63-2	Card and Vit Q, coil	Slightly turbid	Neg	Neg	Neg	Not toxic 10^2	Yes	
63-3	Tape from Vit Q, cap.	Orange, turbid	Rods	B. globigii	Growth	–	No	
64-1	Card and I R C resistors	Clear	Neg	Neg	Neg	Not toxic	Yes	
64-2	Card and Vit Q, coil	Very turbid, green	Rods	B. globigii	Growth	–	No	
64-3	Tape from Vit Q cap.	Clear	Neg	Neg	Neg	Not toxic	Yes	

Table A-6

STERILE ASSEMBLY TECHNIQUES BACTERIOLOGY REPORT
– CANNED IN ETO –

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
75-1	Card and I R C resistors	Turbid, orange	Rods	B. globigii	Growth	–	No	All sterilized for 8 days
75-2	Card and Vit Q, coil	Green and turbid	Neg	Neg	Neg	Not toxic 10^2	Yes	
75-3	Tape from Vit Q cap.	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
76-1	Card and I R C resistors	Sl green, clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
76-2	Card and Vit Q, coil	Sl turbid and green	Neg	Neg	Neg	Not toxic 10^2	Yes	
76-3	Tape from Vit Q cap.	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
77-1	Card and I R C resistors	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
77-2	Card and Vit Q, coil	Turbid, orange	Rods	B. globigii	Growth	–	No	
77-3	Tape from Vit Q	Turbid, orange	Rods	B. globigii	Growth	–	No	
78-1	Card and I R C resistors	Clear, sl green	Neg	Neg	Neg	Not toxic 10^2	Yes	
78-2	Card and Vit Q, coil	Turbid, green	Rods	B. globigii	Growth	–	No	
78-3	Tape from Vit Q	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
79-1	Card and I R C resistors	Clear, sl green	Neg	Neg	Neg	Not toxic 10^2	Yes	
79-2	Card and Vit Q, coil	Clear, sl green	Neg	Neg	Neg	Not toxic 10^2	Yes	
79-3	Tape from Vit Q	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	

Table A-7

STERILE ASSEMBLY TECHNIQUES BACTERIOLOGY REPORT
– ASSEMBLED IN STERILE AIR –

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
47-1	Card and I R C resistors	Sl green, clear	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
47-2	Card and Vit Q cap.	Clear	Neg	Neg	Neg	↓	Yes	
47-3	Tape from Vit Q cap.	Clear	Neg	Neg	Neg		Yes	Sterilized 17 days
48-1	Card and I R C resistors	Turbid, greenish	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
48-2	Card and Vit Q cap.	Green, clear	Neg	Neg	Neg	↓	Yes	
48-3	Tape from Vit Q cap.	Clear	Neg	Neg	Neg		Yes	Sterilized 17 days
49-1	Card and I R C resistors	Green, turbid	Neg (debris)	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
49-2	Card and Vit Q cap.	Greenish, clear	Neg	Neg	Neg		Yes	
49-3	Tape from Vit Q cap.	Turbid, orange	Rods	B. globigii	Growth	–	No	Sterilized 17 days
80-1	Card and I R C resistors	Clear	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
80-2	Card and Vit Q cap.	Sl turbid	Neg	Neg	Neg	↓	Yes	
80-3	Tape from Vit Q cap.	Clear	Neg	Neg	Neg		Yes	Sterilized 12 days
88-1	Card and I R C resistors	Clear	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
88-2	Card and Vit Q cap.	Clear	Neg	Neg	Neg	↓	Yes	
88-3	Tape from Vit Q cap.	Clear	Neg	Neg	Neg		Yes	Sterilized 12 days
67-1	Card made in sterile air (1-part of circuit board, I R C resistors)	Clear, sl green	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
67-2	Piece of circuit board with Vit Q capacitors	Clear, sl green	Neg	Neg	Neg	↓	Yes	
67-3	Tape removed from Vit Q capacitors	Turbid, orange	Rods	B. globigii	Growth		No	Sterilized 12 days

Table A-8

STERILE ASSEMBLY TECHNIQUES BACTERIOLOGY REPORT
– CANNED IN STERILE AIR –

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
58-1	Card and I R C resistors	Turbid, orange	Rods	B. globigii	Growth	–	No	
58-2	Card and Vit Q, coil	Turbid	Rods	B. globigii	Growth	–	No	
58-3	Tape from Vit Q cap.	Clear	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	Sterilized 27 days
66-1	Card and I R C resistors	Sl turbid, green	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
66-2	Card and Vit Q, coil	Turbid	Rods	B. globigii	Growth	–	No	
66-3	Tape from Vit Q cap.	Turbid, orange	Rods	B. globigii	Growth	–	No	Sterilized 27 days
81-1	Card and I R C resistors	Sl green, clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
81-2	Card and Vit Q , coil	Sl green, clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
81-3	Tape from Vit Q cap.	Turbid, orange	Rods	B. globigii	Growth	–	No	Sterilized 14 days
82-1	Card and I R C resistors	Sl green	Neg	Neg	Neg	Not toxic 10^2	Yes	
82-2	Card and Vit Q, coil	Turbid	Rods	B. globigii	Growth	–	No	
82-3	Tape from Vit Q cap.	Turbid, orange	Rods	B. globigii	Growth	–	No	Sterilized 14 days
83-1	Card and I R C resistors	Clear, green	Neg	Neg	Neg	Toxic to 10^4	Inconclusive	Toxic
83-2	Card and Vit Q, coil	Turbid	Rods	B. globigii	Growth	–	No	
83-3	Tape from Vit Q cap.	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	Sterilized 14 days
PS 1	Filter paper spore strip	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	Sterilized 14 days
PS 2	Filter paper spore strip	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
PS 3	Filter paper spore strip	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
GS 1	Glass slide spore strip	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	Sterilized 14 days
GS 2	Glass slide spore strip	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	

Table A-9

STERILE ASSEMBLY TECHNIQUES BACTERIOLOGY REPORT
– ASSEMBLED IN STERILE NITROGEN –

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
68-1	Card and IRC resistors	Sl green and turbid	Neg	Neg	Neg	Not toxic to 10^2 inocu.	Yes	
-2	Card and Vit Q cap.	Clear	Neg	Neg	Neg	Not toxic to 10^2 inocu.	Yes	Sterilized 20 days
-3	Tape from Vit Q cap.	Clear	Neg	Neg	Neg	Not toxic to 10^2 inocu.	Yes	
69-1	Card and IRC resistors	Sl green and turbid	Neg	Neg	Neg	Not toxic to 10^2 inocu.	Yes	
-2	Card and Vit Q cap.	Sl green	Neg	Neg	Neg	Not toxic to 10^2 inocu.	Yes	
-3	Tape from Vit Q cap.	Clear	Neg	Neg	Neg	Not toxic to 10^2 inocu.	Yes	Sterilized 20 days
85-1	Card and IRC resistors	Mod turbidity	Neg	Neg	Neg	Not toxic to 10^2 inocu.	Yes	
-2	Card and Vit Q cap.	Very turbid, gray green	Heavy debris	Neg	Neg	Not toxic to 10^2 inocu.	Yes	
-3	Tape from Vit Q cap.	Clear	Neg	Neg	Neg	Not toxic to 10^2 inocu.	Yes	Sterilized 14 days
98-1	Board and IRC resistors	Mod turbid, gray green	Neg	Neg	Neg	Not toxic to 10^2 inocu.	Yes	Sterilized 14 days
-2	Card and Vit Q cap.	Mod turbid gray green	Neg	Neg	Neg	Not toxic to 10^2 inocu.	Yes	
73-1	Piece of circuit board and IRC resistors	Clear, sl green	Neg	Neg	Neg	Not toxic to 10^2 inocu.	Yes	Sterilized 20 days
-2	Piece of circuit board and Vit Q capacitors	Clear, sl green	Neg	Neg	Neg	Not toxic to 10^2 inocu.	Yes	
-3	Tape removed from Vit Q capacitors	Clear, no change	Neg	Neg	Neg	Not toxic to 10^2 inocu.	Yes	
71-1	Card and IRC resistors	Sl green, clear	Neg	Neg	Neg	Not toxic to 10^2 inocu.	Yes	Sterilized 20 days
-2	Card and Vit Q cap.	Sl green, clear	Neg	Neg	Neg	Not toxic to 10^2 inocu.	Yes	
-3	Tape from Vit Q cap.	Turbid, orange	Rods	B. globigii	Growth	–	No	

Table A-10

STERILE ASSEMBLY TECHNIQUES BACTERIOLOGY REPORT
– CANNED IN STERILE NITROGEN –

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
74-1	Card and IRC resistors	Turbid, orange	Rods	B. globigii	Growth	–	No	Sterilized 20 days
74-2	Card and Vit Q, coil	Sl, turbid	Neg	Neg	Neg	Not toxic 10^2	Yes	
74-3	Tape from Vit Q	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
72-1	Card and IRC resistors	Sl turbid and green	Neg	Neg	Neg	Not toxic 10^2	Yes	Sterilized 20 days
72-2	Card and Vit Q, and coil	Sl turbid and green	Neg	Neg	Neg	Not toxic 10^2	Yes	
72-3	Tape from Vit Q	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
84-1	Card and IRC resistors	Green, turbid	Neg	Neg	Neg	Not toxic 10^2	Yes	Sterilized 14 days
84-2	Card and Vit Q and coil	Green, turbid	Neg	Neg	Neg	Not toxic 10^2	Yes	
84-3	Tape from Vit Q	Turbid, orange	Rods	B. globigii	Growth	–	No	
86-1	Card and IRC resistors	Sl turbid, green	Neg	Neg	Neg	Not toxic 10^2	Yes	Sterilized 14 days
86-2	Card and Vit Q, coil	Turbid, green	Rods	B. globigii	Growth	–	No	
86-3	Tape from Vit G	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
87-1	Card and IRC resistors	Clear, green	Neg	Neg	Neg	Toxic to 10^4	Inconclusive, toxic	Sterilized 14 days
87-2	Card and Vit Q, coil	Sl turbid, green	Neg	Neg	Neg	Not toxic 10^2	Yes	
87-3	Tape from Vit Q	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	

Table A-11

STERILE ASSEMBLY TECHNIQUES BACTERIOLOGY REPORT
– AIR AND NITROGEN SPORE STRIPS –

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
SA 1	Spore strips	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	Sterilized 12 days
SA 2	Spore strips	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	Sterilized 12 days
SN 1	Spore strips	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	Sterilized 14 days

Table A-12

STERILE ASSEMBLY TECHNIQUES BACTERIOLOGY REPORT
– ASSEMBLED IN ROOM AIR ON BENCH, SURFACE STERILIZED IN GLOVE BOX
(CARDS NOT INOCULATED) –

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
18-1	Card and I R C resistors	Sl green, clear	Neg	Neg	Neg	Not toxic 10^2	Yes	All sterilized for 24 days
18-2	Card and Vit Q cap	Sl green, clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
18-3	Tape from Vit Q cap.	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
19-1	Card and I R C resistors	Sl green, clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
19-2	Card and Vit Q cap.	Sl green, sl turbid	Neg	Neg	Neg	Not toxic 10^2	Yes	
19-3	Tape from Vit Q cap.	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
20-1	Card and I R C resistors	Sl green, clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
20-2	Card and Vit Q cap.	Sl green, clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
20-3	Tape from Vit Q cap.	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
21-1	Card and I R C resistors	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
21-2	Card and Vit Q cap.	Sl green, sl turbid	Neg	Neg	Neg	Not toxic 10^2	Yes	
21-3	Tape from Vit Q cap.	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
22-1	Card and I R C resistors	Clear, sl green	Neg	Neg	Neg	Not toxic 10^2	Yes	
22-2	Card and Vit Q, coil	Clear, sl green	Neg	Neg	Neg	Not toxic 10^2	Yes	
22-3	Tape from Vit Q cap.	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	

Table A-13

STERILE ASSEMBLY TECHNIQUES BACTERIOLOGY REPORT – ASSEMBLED AND TESTED ON BENCH, PLACED IN CANS, SURFACE STERILIZED IN GLOVE BOX (CARDS NOT INOCULATED) –

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
13-1	Card and I R C resistors	Clear. sl green	Neg	Neg	Neg	Not toxic 10^2	Yes	
13-2	Card and Vit Q. coil	Turbid. sl green	Neg	Neg	Neg	Not toxic 10^2	Yes	
13-3	Tape from Vit Q	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
14-1	Card and I R C resistors	Clear. green	Neg	Neg	Neg	Toxic to 10^4	Inconclusive	Toxic
14-2	Card and Vit Q. coil	Clear. green	Neg	Neg	Neg	Toxic to 10^4	Inconclusive	Toxic
14-3	Tape from Vit Q	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
15-1A	Card and I R C resistors	Clear, green	Neg	Neg	Neg	Not toxic 10^2	Yes	
15-1B	Card 2 I R C, diode	Clear. green	Neg	Neg	Neg	Toxic to 10^2	Inconclusive	Toxic (in 60 ml)
15-2	Card and Vit Q, coil	Clear. green	Neg	Neg	Neg	Not toxic 10^2	Yes	
15-3	Tape from Vit Q	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
16-1	Card and I R C resistors	Clear, sl green	Neg	Neg	Neg	Not toxic 10^2	Yes	
16-2	Card and Vit Q, coil	Clear. sl green	Jar broken when opened for testing (7 days) Gross appearance – Negative					
16-3	Tape from Vit Q	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
17-1	Card and I R C resistors	Sl green and turbid	Neg	Neg	Neg	Not toxic 10^2	Yes	
17-2	Card and Vit Q, coil	Sl green and turbid	Neg	Neg	Neg	Not toxic 10^2	Yes	
17-3	Tape from Vit Q	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	

Table A-14

STERILE ASSEMBLY TECHNIQUES BACTERIOLOGY REPORT
– STERILIZATION SKIP INVESTIGATION TEST NO. 1 –

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
C006	Bottled in large box (I R C)	Clear, no color change	Neg	Neg	Neg	Not toxic 10^2 in 100ml	Yes	
C006	Bottled in large box (I R C)	Clear, no color change	Neg	Neg	Neg	Not toxic 10^2 in 100ml	Yes	
F006	Bottled in large box (Vit Q)	Turbid	Rods	B. globigii	Growth	–	No	Contaminated in 24 hr Sterilized 5 days
K006	Bottled in small box (Small black diode)	Clear, no color change	Neg	Neg	Neg	Not toxic 10^2 in 100ml	Yes	
PC006	Printed circuit board	Turbid, brown-gray color	Rods	B. globigii	Growth	–	No	
A006	Inductance coil	Clear, no change	Neg	Neg	Neg	Not toxic 10^2 in 100ml	Yes	
J006	Vit Q	Turbid	Rods	B. globigii	Growth	–	No	Contaminated in 48 hr
I006	I R C	Clear	Neg	Neg	Neg	Not toxic 10^2 in 100ml	Yes	
B006	Vit Q	Turbid	Rods	B. globigii	Growth	–	No	Contaminated in 24 hr
H006	3 lead transistor	Turbid	Rods	B. globigii	Growth	–	No	Contaminated in 24 hr
L006	I R C	Clear	Neg	Neg	Neg	Not toxic 10^2 in 100ml	Yes	
Nut-Bolt 006	Not inoc.	Turbid	Inconclusive	Neg	Neg	↓	Yes	Sterilized 5 days
Air	From cart to small box	Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
D006 and	Bottled in small box	Turbid	Rods	B. globigii	Growth	–	No	Contaminated in 24 hr
G006	(Vit Q and I R C)	Turbid	Rods	B. globigii	Growth	–	No	Sterilized 5 days
A009	Induction coil	Turbid	Rods	B. globigii	Growth	–	No	
B009	Vit Q capacitor	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 in 100ml	Yes	
F009	Vit Q capacitor	↓	Neg	Neg	Neg	↓	Yes	
G009	Vit Q capacitor	Turbid	Rods	B. globigii	Growth	–	No	Sterilized 5 days
H009	Transistor – 3 lead	Clear	Neg	Neg	Neg	Toxic to 10^2 in 15 ml	Inconclusive	Sterilized 5 days This component is toxic to 10^2 inoc. in 15 ml of culture medium, but not in 100 ml.
J009	Vit Q capacitor	Turbid	Rods	B. globigii	Growth	–	No	Sterilized 5 days
K009	Diode, small black	Clear	Neg	Neg	Neg	Not toxic to 10^2 in 15 ml	Yes	

Table A-15

STERILE ASSEMBLY TECHNIQUES BACTERIOLOGY REPORT
– STERILIZATION SKIP INVESTIGATION TEST NO. 2 –

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
PC004	Two pieces circuit board with soldered tracks	Gray-black very turbid	Neg	Neg	Neg	Growth with 10^2 inoc. in 100 ml	Yes	All sterilized for 5 days
B004	Vitamin Q Cap.	Clear	Neg	Neg	Neg	Growth 10^2 inoc. in 100 ml	Yes	
C004	IRC resistor	Clear	Neg	Neg	Neg	Growth 10^2 inoc. in 100 ml	Yes	
D004	IRC resistor	Sl cloudy	Neg	Neg	Neg	Very scant growth in 10 ml	Yes	
E004	IRC resistor	Sl cloudy	Neg	Neg	Neg	No growth in 10 ml. 10^2	Inconclusive	Probably toxic
F004	Vit. Q capacitor	Clear	Neg	Neg	Neg	Growth in 100 ml, 10^2 inoc.	Yes	
G004	Vit. Q capacitor	Clear	Neg	Neg	Neg	No growth in 100 ml	Inconclusive	Probably toxic
H004	Transistor, 3 lead	Clear	Neg	Neg	Neg	Doubtful growth	Yes	
I004	IRC resistor	Clear	Neg	Neg	Neg	Growth in 10 ml	Yes	
J004	Vit. Q capacitor	Cloudy, sediment	Neg	Neg	Neg	Scant growth	Yes	
K004	Diode, small black	Cloudy, sediment	Neg	Neg	Neg	Growth in 10 ml	Yes	
L004	IRC resistor	Clear	Neg	Neg	Neg	Growth in 10 ml	Yes	
C009	IRC resistor	Clear	Neg	Neg	Neg	Growth in 10 with 10^2 inoc.	Yes	
D009	IRC resistor	Clear	Neg	Neg	Neg	No growth 10 ml	Inconclusive	
E009	IRC resistor	Clear	Neg	Neg	Neg	Scant growth	Yes	
I009	IRC resistor	Clear	Neg	Neg	Neg	No growth in 10 ml	Inconclusive	

Table A-15 (Cont.)

Jar No.	Assembly Step and Description	Culture Media Apperance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
L009	IRC resistor	Clear	Neg	Neg	Neg	No growth in 10 ml	Inconclusive	All sterilized for 5 days
PC007	1/3 of circuit board containing IRC resistor and coil	Clear	Neg	Neg	Neg	Very scant growth-100 ml	Yes	
PC007	1/3 circuit board containing 3 Vit. Q cap. and diode	Clear	Neg	Neg	Neg	No growth in 100 ml media with 10^2 inoc.	Inconclusive	
PC007	1/3 circuit board 5 IRC cap.	Sl green	Neg	Neg	Neg	No growth	Inconclusive	
PC009	1/2 plain circuit board	Sl turbid	Neg	Neg	Neg	No growth	Inconclusive	Toxic
PC009	1/2 plain circuit board	Turbid	Rods	B. globigii	Contaminated	–	No	Contaminated in 48 hr
ST 1	Spore strip containing 100 B. globigii spores transferred to culture media in box.	Turbid	Rods	B. globigii	Contaminated	–	No	
ST 2	Spore strip containing 100 B. globigii spores transferred to culture media in box.	Clear	Neg	Neg	Neg	Growth with 10^2 inoculum	Yes	
	Finger of glove dipped in jar of culture media.	Clear	Neg	Neg	Neg	Growth with 10^2 inoc.	Yes	

Table A-16

STERILE ASSEMBLY TECHNIQUES BACTERIOLOGY REPORT – STERILIZATION SKIP INVESTIGATION TEST NO. 3 –

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
CO								
1	Induction coil	Very slight turbidity	Neg	Neg	Neg	Not toxic to 10^2 inoc.	Yes	
2	Induction coil	Sl turbidity	Neg	Neg	Neg	Not toxic to 10^2 inoc.	Yes	
3	Induction coil	Sl turbidity	Neg	Neg	Neg	Not toxic to 10^2 inoc.	Yes	
4	Induction coil	Dark gray, very turbid	Heavy debris no bacteria	Neg	Neg	Not toxic to 10^2 inoc.	Yes	
CA								
1	Bottled 5 Vit Q capacitors	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoc.	Yes	
2	Bottled 5 Vit Q capacitors	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoc.	Yes	
3	Bottled 5 Vit Q capacitors	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoc.	Yes	
4	Bottled 5 Vit Q capacitors	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoc.	Yes	
5	Bottled 5 Vit Q capacitors	Turbid, orange color	Rods	B. Globigii	Growth	–	No	Contaminated 24 hr
SS-C	Bottled 2 spore strips	Clear	Neg	Neg	Neg	Not toxic to 10^2 inoc.	Yes	
–	Cotton from STA-1 Flask-Air	Clear	Neg	Neg	Neg	Not toxic to 10^2 inoc.	Yes	Sterilized for 4 days

Table A-17

STERILE ASSEMBLY TECHNIQUES BACTERIOLOGY REPORT
– ADDITIONAL CAPACITOR CHECK –

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
CTR 1	Vit Q capacitors with PS tape removed	Clear, no change	Neg	Neg	Neg	Not toxic 10^2 inoculum	Yes	
2	Vit Q capacitors with PS tape removed	Clear, no change	Neg	Neg	Neg	Not toxic 10^2 inoculum	Yes	
3	Vit Q capacitors with PS tape removed	Clear, no change	Neg	Neg	Neg	Not toxic 10^2 inoculum	Yes	
4	Vit Q capacitors with PS tape removed	Clear, no change	Neg	Neg	Neg	Not toxic 10^2 inoculum	Yes	
5	Vit Q capacitors with PS tape removed	Clear, no change	Neg	Neg	Neg	Not toxic 10^2 inoculum	Yes	
6	Vit Q capacitors with PS tape removed	Clear, no change	Neg	Neg	Neg	Not toxic 10^2 inoculum	Yes	
TA 1	Tape removed from above capacitors	Turbid, orange color	Rods	B. globigii	Growth	–	No	Contaminated for 3 days
TA 2	Tape removed from 5 additional capacitors in MGB	Clear, no color change	Neg	Neg	Neg	Not toxic 10^2 inoculum	Yes	Sterilized for 6 days

Table A-18

STERILE ASSEMBLY TECHNIQUES BACTERIOLOGY REPORT
– STERILITY CHECK – (STRIPPED AND NONSTRIPPED CAPACITORS) –

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
1 T	5 capacitors with plastic tape cover (Vit Q type)	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
2 T	5 capacitors with plastic tape cover (Vit Q type)	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
3 T	5 capacitors with plastic tape cover (Vit Q type)	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
4 T	5 capacitors with plastic tape cover (Vit Q type)	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
5 T	5 capacitors with plastic tape cover (Vit Q type)	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	Sterilized 3 days
1 R	5 capacitors with plastic tape removed (Vit Q)	Turbid, orange color	Some rods, some appear to be strep and cocci	All colonies appear typical of B. globigii	Growth	–	No	Contaminated in 48 hr
2 R	5 capacitors with plastic tape removed (Vit Q)	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
3 R	5 capacitors with plastic tape removed (Vit Q)	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
4 R	(2 capacitors in same bottle)	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	Sterilized 3 days
1ssc	1 Spore Strip control	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	Sterilized 3 days

Table A-19

STERILE ASSEMBLY TECHNIQUES BACTERIOLOGY REPORT
– INOCULATION TECHNIQUE TEST –

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
W 1	5 capacitors (Vit Q) dipped in water spore solution	Turbid, orange color	Rods	B. globigii	Growth	–	No	
W 2	5 capacitors (Vit Q) dipped in water spore solution	Turbid, orange color	Rods	B. globigii	Growth	–	No	
W 3	5 capacitors (Vit Q) dipped in water spore solution	Turbid, orange color	Rods	B. globigii	Growth	–	No	
W 4	5 capacitors (Vit Q) dipped in water spore solution	Turbid, orange color	Rods	B. globigii	Growth	–	No	
W 5	5 capacitors (Vit Q) dipped in water spore solution	Turbid, orange	Rods	B. globigii	Growth	–	No	Sterilized 24 hr
A 1	5 capacitors dipped in acetone spore suspension (Vit Q)	Turbid, orange	Rods	B. globigii	Growth	–	No	
A 2	5 capacitors dipped in acetone spore suspension (Vit Q)	Turbid, orange color	Rods	B. globigii	Growth	–	No	
A 3	5 capacitors dipped in acetone spore suspension (Vit Q)	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
A 4	5 capacitors dipped in acetone spore suspension (Vit Q)	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
A 5	5 capacitors dipped in acetone spore suspension (Vit Q)	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	Sterilized 24 hr
AP 1	5 Vit Q capacitors with acetone spore solution applied by pipette	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
AP 2	5 Vit Q capacitors with acetone spore solution applied by pipette	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
AP 3	5 Vit Q capacitors with acetone spore solution applied by pipette	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes Yes	
AP 4	5 Vit Q capacitors with acetone spore solution applied by pipette	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
AP 5	5 Vit Q capacitors with acetone spore solution applied by pipette	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	Sterilized 24 hr

Table A-19 (Cont.)

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
W 6	5 Vit Q capacitors dipped in water spore suspension	Clear, no change	Neg	Neg	Neg	Not toxic 10^2	Yes	
W 7	5 Vit Q capacitors dipped in water spore suspension	Turbid, orange	Rods	B. globigii	Growth	–	No	
W 8	5 Vit Q capacitors dipped in water spore suspension	Turbid, orange	Rods	B. globigii	Growth	–	No	
W 9	5 Vit Q capacitors dipped in water spore suspension	Clear, no change	Neg	Neg	Neg	Not toxic 10^2	Yes	
W10	5 Vit Q capacitors dipped in water spore suspension	Turbid, orange	Rods	B. globigii	Growth	–	No	Sterilized 48 hr
A 6	5 Vit Q capacitors dipped in acetone spore solution	Clear, no change	Neg	Neg	Neg	Not toxic 10^2	Yes	
A 7	5 Vit Q capacitors dipped in acetone spore solution	Clear, no change	Neg	Neg	Neg	Not toxic 10^2	Yes	
A 8	5 Vit Q capacitors dipped in acetone spore solution	Turbid, orange	Rods	B. globigii	Growth	–	No	
A 9	5 Vit Q capacitors dipped in acetone spore solution	Turbid, orange	Rods	B. globigii	Growth	–	No	
A 10	5 Vit Q capacitors dipped in acetone spore solution	Clear, no change	Neg	Neg	Neg	Not toxic 10^2	Yes	Sterilized 48 hr
AP 6	5 Vit Q capacitors with acetone spore suspension applied with pipette	Clear, no change	Neg	Neg	Neg	Not toxic 10^2	Yes	
AP 7	5 Vit Q capacitors with acetone spore suspension applied with pipette	Turbid, orange	Rods	B. globigii	Growth	–	No	
AP 8	5 Vit Q capacitors with acetone spore suspension applied with pipette	Clear, no change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
AP 9	5 Vit Q capacitors with acetone spore suspension applied with pipette	Clear, no change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
AP 10	5 Vit Q capacitors with acetone spore suspension applied with pipette	Clear, no change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	Sterilized 48 hr

Table A-19 (Cont.)

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree Competent Toxicity	Competent Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
W 11	5 Vit Q capacitors dipped in water spore solution	Turbid, orange	Rods	B. globigii	Growth	–	No	
W 12	5 Vit Q capacitors dipped in water spore solution	Turbid, orange	Rods	B. globigii	Growth	–	No	
W 13	5 Vit Q capacitors dipped in water spore solution	Turbid, orange	Rods	B. globigii	Growth	–	No	
W 14	5 Vit Q capacitors dipped in water spore solution	Turbid, orange	Rods	B. globigii	Growth	–	No	
W 15	5 Vit Q capacitors dipped in water spore solution	Turbid, orange	Rods	B. globigii	Growth	–	No	Sterilized 120 hr
A 11	5 Vit Q capacitors dipped in acetone spore solution	Turbid, orange	Rods	B. globigii	Growth	–	No	
A 12	5 Vit Q capacitors dipped in acetone spore solution	Clear, no change	Neg	Neg	Neg	Not toxic 10^2 inoculum	Yes	
A 13	5 Vit Q capacitors dipped in acetone spore solution	Clear, no change	Neg	Neg	Neg	Not toxic 10^2 inoculum	Yes	
A 14	5 Vit Q capacitors dipped in acetone spore solution	Clear, no change	Neg	Neg	Neg	Not toxic 10^2 inoculum	Yes	
A 15	5 Vit Q capacitors dipped in acetone spore solution	Turbid, orange	Rods	B. globigii	Growth	–	No	Sterilized 120 hr
AP 11	5 Vit Q capacitors with acetone spore solution applied with pipette	Turbid, orange	Rods	B. globigii	Growth	–	No	
AP 12	5 Vit Q capacitors with acetone spore solution applied with pipette	Clear, no change	Neg	Neg	Neg	Not toxic 10^2 inoculum	Yes	
AP 13	5 Vit Q capacitors with acetone spore solution applied with pipette	Clear, no change	Neg	Neg	Neg	Not toxic 10^2 inoculum	Yes	
AP 14	5 Vit Q capacitors with acetone spore solution applied with pipette	Clear, no change	Rods	B. globigii	Growth	–	No	
AP 15	5 Vit Q capacitors with acetone spore solution applied with pipette	Clear, no change	Neg	Neg	Neg	Not toxic 10^2 inoculum	Yes	Sterilized 120 hr

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
W 16	5 Vit Q capacitors dipped in water spore solution	Turbid, orange color	Rods	B. globigii	Growth	–	No	
W 17	5 Vit Q capacitors dipped in water spore solution	Turbid, orange color	Rods	B. globigii	Growth	–	No	
W 18	5 Vit Q capacitors dipped in water spore solution	Turbid, orange color	Rods	B. globigii	Growth	–	No	
W 19	5 Vit Q capacitors dipped in water spore solution	Turbid, orange color	Rods	B. globigii	Growth	–	No	
W 20	5 Vit Q capacitors dipped in water spore solution	Turbid, orange color	Rods	B. globigii	Growth	–	No	Sterilized 144 hr
A 16	5 Vit Q capacitors dipped in acetone spore solution	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
A 17	5 Vit Q capacitors dipped in acetone spore solution	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
A 18	5 Vit Q capacitors dipped in acetone spore solution	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
A 19	5 Vit Q capacitors dipped in acetone spore solution	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
A 20	5 Vit Q capacitors dipped in acetone spore solution	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	Sterilized 144 hr
AP 16	5 Vit Q capacitors with acetone spore suspension applied by pipette	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
AP 17	5 Vit Q capacitors with acetone spore suspension applied by pipette	Clear, nc color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
AP 18	5 Vit Q capacitors with acetone spore suspension applied by pipette	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
AP 19	5 Vit Q capacitors with acetone spore suspension applied by pipette	Turbid, orange	Rods	B. globigii	Growth	–	No	
AP 20	5 Vit Q capacitors with acetone spore suspension applied by pipette	Clear, no change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	Sterilized 144 hr
GS 3	Glass slide spore strip	Clear, no change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
GS 4	Glass slide spore strip	Clear, no change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	Sterilized 144 hr

Table A-20

STERILE ASSEMBLY TECHNIQUES BACTERIOLOGY REPORT
– INVESTIGATION OF SURFACE EFFECTS –

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer Agar Plate	Tube of Broth	Degree of Component Toxicity	Component Sterile Yes/No	Remarks
38	Four aluminum plates coated with Scotch tape	Turbid, orange	Rods	B. globigii	Growth	–	No	
82	↓	↓	Rods	B. globigii	Growth	–	No	
83			Rods	B. globigii	Growth	–	No	
84			Rods	B. globigii	Growth	–	No	Sterilized 9 days
37	Four aluminum plates, uncoated	Turbid, orange	Rods	B. globigii	Growth	–	No	
73	↓	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
75		Turbid, orange	Rods	B. globigii	Growth	–	No	
76		Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	Sterilized 9 days
74-81	Two plates, one coated, one not coated	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	Sterilized 9 days
SSP	One spore strip	Clear, no color change	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	Sterilized 9 days

Table A-21

STERILE ASSEMBLY TECHNIQUES BACTERIOLOGY REPORT
– 100 PERCENT ETHYLENE OXIDE –

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
CHO								
1	Vit Q capacitors processed in 100% ethylene oxide	Turbid, orange color	Rods Rods	B. globigii	Growth	–	No	
2	↓	↓	Rods	B. globigii	Growth	–	No	
3			Rods	B. globigii	Growth	–	No	
4			Rods	B. globigii	Growth	–	No	
5			Rods	B. globigii	Growth	–	No	
6			Rods	B. globigii	Growth	–	No	
7			Rods	B. globigii	Growth	–	No	
8			Rods	B. globigii	Growth	–	No	
9			Rods	B. globigii	Growth	–	No	
10			Rods	B. globigii	Growth	–	No	Sterilized 6-1/2 hr

Table A-21 (Cont.)

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
CHO	Capacitors soaked in 100% ETO							
30	1 Vit Q capacitor	Turbid, orange color	Rods	B. globigii	Growth	Not applicable	No	
31	↓	↓	Rods	B. globigii	Growth	↓	No	
32			Rods	B. globigii	Growth		No	
33			Rods	B. globigii	Growth		No	
34			Rods	B. globigii	Growth		No	
35			Rods	B. globigii	Growth		No	
36			Rods	B. globigii	Growth		No	
37			Rods	B. globigii	Growth		No	
38			Rods	B. globigii	Growth		No	
39			Rods	B. globigii	Growth		No	Sterilized 54 hr

Table A-21 (Cont.)

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
CHO	Capacitors soaked in 100% ETO							
11	1 Vit Q capacitor	Clear	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
12		Turbid, orange color	Rods	B. globigii	Growth	–	No	
13		Turbid, orange color	Rods	B. globigii	Growth	–	No	
14		Turbid, orange color	Rods	B. globigii	Growth	–	No	
15		Clear	Neg	Neg	Neg	Not toxic to 10^2 inoculum	Yes	
16		Turbid, orange color	Rods	B. globigii	Growth	–	No	
17		Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
18		Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
19		Clear	Neg	Neg	Neg	Not toxic 10^2	Yes	
20		Turbid, orange color	Rods	B. globigii	Growth	–	No	
21		Turbid, orange color	Rods	B. globigii	Growth	–	No	Sterilized 60 hr
1	Jar of cotton from filter	Clear	Neg	Neg	Neg	Not toxic	Yes	

Table A-22

STERILE ASSEMBLY TECHNIQUES BACTERIOLOGY REPORT – REHYDRATION TEST

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
WDT								
1	Water dipped. sterilized in tube	Clear. no change	Neg	Neg	Neg	Not toxic 10^2	Yes	
2		Turbid. orange	Rods	B. globigii	Growth	–	No	
3		Clear. no change	Neg	Neg	Neg	Not toxic 10^2	Yes	
4			Neg	Neg	Neg		Yes	
5			Neg	Neg	Neg		Yes	
6			Neg	Neg	Neg		Yes	
7			Neg	Neg	Neg		Yes	
8			Neg	Neg	Neg		Yes	
9		Turbid, orange	Rods	B. globigii	Growth	–	No	Sterilized 83 hr
10		Clear, no change	Neg	Neg	Neg	Not toxic 10^2	Yes	
WAT								
1	Water added. sterilized in tube	Clear, no change	Neg	Neg	Neg	Not toxic 10^2	Yes	
2			Neg	Neg	Neg		Yes	
3			Neg	Neg	Neg		Yes	
4			Neg	Neg	Neg		Yes	
5			Neg	Neg	Neg		Yes	
6			Neg	Neg	Neg		Yes	
7			Neg	Neg	Neg		Yes	
8			Neg	Neg	Neg		Yes	
9			Neg	Neg	Neg		Yes	Sterilized
10			Neg	Neg	Neg		Yes	83 hr
WDB								
1	Water dipped. sterlized in glove box	Clear, no change	Neg	Neg	Neg	Not toxic 10^2	Yes	
2			Neg	Neg	Neg		Yes	
3			Neg	Neg	Neg		Yes	
4			Neg	Neg	Neg		Yes	
5			Neg	Neg	Neg		Yes	
6			Neg	Neg	Neg		Yes	
7			Neg	Neg	Neg		Yes	
8			Neg	Neg	Neg		Yes	
9			Neg	Neg	Neg		Yes	Sterilized
10			Neg	Neg	Neg		Yes	83 hr

Table A-22 (Cont.)

Jar No.	Assembly Step and Description	Culture Media Appearance After 7 Days Incubation	Microscopic Examination	Check for Growth by Transfer		Degree of Component Toxicity	Component Sterile Yes/No	Remarks
				Agar Plate	Tube of Broth			
WAB								
1	Water added, sterilized in glove box	Clear, no change	Neg	Neg	Neg	Not toxic 10^{-2}	Yes	
2			Neg	Neg	Neg	Not toxic 10^{-2}	Yes	
3			Neg	Neg	Neg	Not toxic 10^{-2}	Yes	
4			Neg	Neg	Neg	Not toxic 10^{-2}	Yes	
5			Neg	Neg	Neg	Not toxic 10^{-2}	Yes	
6			Neg	Neg	Neg	Not toxic 10^{-2}	Yes	
7			Neg	Neg	Neg	Not toxic 10^{-2}	Yes	
8			Neg	Neg	Neg	Not toxic 10^{-2}	Yes	
9			Neg	Neg	Neg	Not toxic 10^{-2}	Yes	Sterilized 83 hr
10			Neg	Neg	Neg	Not toxic 10^{-2}	Yes	
W 1	Paper spore strip in tube	Clear, no change	Neg	Neg	Neg	Not toxic 10^{-2}	Yes	
W 2	Glass spore strip in glove box	Clear, no change	Neg	Neg	Neg	Not toxic 10^{-2}	Yes	
W 3	Wet glass in tube	Clear, no change	Neg	Neg	Neg	Not toxic 10^{-2}	Yes	
W 4	Dry in glove box	Clear, no change	Neg	Neg	Neg	Not toxic 10^{-2}	Yes	
W 5	Paper spore strip in box	Clear, no change	Neg	Neg	Neg	Not toxic 10^{-2}	Yes	
W 6	Dry glass in tube	Turbid, orange	Rods	B. globigii	Growth	–	No	Sterilized 83 hr